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GLYCOSIDES OF 2-PHENYLETHANOL AND BENZYL ALCOHOL IN *VITIS VINIFERA* GRAPES

PATRICK J. WILLIAMS, CHRISTOPHER R. STRAUSS, BEVAN WILSON and RALPH A. MASSY-WESTROPP*

The Australian Wine Research Institute, Private Mail Bag, Glen Osmond, South Australia 5064, Australia; *Department of Organic Chemistry, University of Adelaide, P. O. Box 498, Adelaide, South Australia 5001 Australia

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Abstract—β-Rutinosides and 6-*O*-α-L-arabinofuranosyl-β-D-glucopyranosides of 2-phenylethanol and benzyl alcohol have been found to co-occur with similar disaccharide glycosides of monoterpenes in *Vitis vinifera* vars. Muscat of Alexandria and Rhine Riesling. β-D-Glucopyranosides of these two alcohols were also identified in the fruit.

INTRODUCTION

In the course of studies on the isolation of monoterpene glycosides from *Vitis vinifera* grapes, precursors of 2-phenylethanol and benzyl alcohol were also observed in the fruit [1]. Subsequent work on the linalol oxidation state monoterpene glycosides showed these to be a mixture of β-rutinosides and 6-*O*-α-L-arabinofuranosyl-β-D-glucopyranosides of predominantly geraniol, nerol and linalol together with smaller amounts of α-terpineol [2]. This paper reports that the 2-phenylethanol and benzyl alcohol precursors in the grapes exist as a mixture of β-D-glucopyranosides and the above diglycosides.

RESULTS AND DISCUSSION

Hydrolysis at pH 1.0 of a glycosidic fraction isolated by prep. C_{18}-reversed phase chromatography from Muscat of Alexandria grape juice or wine gave both 2-phenylethanol and benzyl alcohol (**1a**, **2a**) [1]. Enzymatic hydrolysis of the reversed phase material using a commercial pectinase preparation, Rohapect C, at pH 5 also gave these two alcohols. This enzyme preparation had been used to liberate monoterpene aglycones from diglycosides present in the same source material [2]. Reversed phase isolates from different samples of both Muscat of Alexandria and Rhine Riesling grape juices all gave the alcohols (**1a**, **2a**) on enzymatic hydrolysis after prior extraction of the substrates to ensure removal of the free alcohols. Also, it was observed that the proportions of compounds **1a** and **2a** given in these experiments varied with the maturity of the fruit from which the reversed phase material was isolated. Maximum yields were obtained with ripe fruit. Thus, for example, a sample of Muscat of Alexandria juice of 20.8° Brix gave, on reversed phase chromatography, a precursor concentrate which yielded alcohols **1a** and **2a** in a ratio of 2:1. Furthermore, they were produced in approximately 10% of the amount of the geraniol released by the glycosidase activity of Rohapect C.

These data suggested that 2-phenylethanol (**1a**) and benzyl alcohol (**2a**) were present in grapes as glycosides. To investigate the sugar moieties, GC analyses were made of the acetylated reversed phase material, and fractions of this after chromatography on Si gel. Additionally, the reversed phase concentrate, both before and after prep. TLC, was trimethylsilylated for GC studies. Because of the complexity of all of these fractions, which included glycosides of monoterpenes at different oxidation states and glycosides of nor-isoprenoid compounds together with the benzyl and 2-phenylethyl derivatives, the isolation of individual compounds was not practicable. Accordingly, capillary column GC and GC/MS analyses were necessary for structural assignment.

Authentic 2,3,4,6-tetra-*O*-acetyl β-D-glucopyranosides of 2-phenylethanol and benzyl alcohol (**1c** and **2c**) were synthesized under Koenigs–Knorr conditions and used as reference materials. EIMS of the 2-phenylethyl compound (**1c**) showed characteristic fragments for the aglycone at *m/z* 105 $[C_6H_5CH_2CH_2]^+$ and *m/z* 104 $[C_6H_5CH{=}CH_2]^+$ as well as ions anticipated for the acetylated glucose moiety at *m/z* 331, 169 and 109 [3, 4]. Synthetic benzyl compound (**2c**) gave *m/z* 91 $[C_6H_5CH_2]^+$ for the aglycone but, in addition to the expected fragments for the acetylated glucose portion, prominent ions at *m/z* 152.0471 $[C_8H_8O_3]^+$, 139.0399 $[C_7H_7O_3]^+$, 110.0370 $[C_6H_6O_2]^+$, 97.0289 $[C_5H_5O_2]^+$ were observed. These latter ions, which are seldom prominent if observed at all in EIMS of 2,3,4,6-tetra-*O*-acetyl glucopyranosides [3–6], appear to have been formed from (**2c**) by elimination of acetic acid and benzaldehyde from the molecular ion, leading to a 3,4,6-tri-*O*-acetyl glucal (MW 272) which, subsequently, fragmented. Significantly, in the EIMS of 3,4,6-tri-*O*-acetyl glucal (**3**) itself these ions are very prominent [7]. The presence of these triacetyl glucal-derived ions was particularly diagnostic and served to characterize acetylated benzyl glycosides.

GC/MS of the acetylated reversed phase material showed the presence of **1c** and **2c** and these were confirmed by cochromatography with the synthetic reference compounds. Thus, the β-D-glucopyranosides (**1b** and **2b**) occur naturally in the grape material.

Examination of the longer R_t components in the

acetylated fractions showed peaks corresponding to two diglycosides of each alcohol (**1a** and **2a**). These four compounds emerged in the chromatographic region in which the co-occurring acetylated β-rutinosides and 6-*O*-α-L-arabinofuranosyl-β-D-glucopyranosides of several monoterpenes [2] appeared. EIMS of the longer R_t of the two 2-phenylethyl disaccharide acetates showed, in addition to the aglycone fragments *m/z* 105 and 104, prominent ions at *m/z* 259 and 139. These latter fragments are derived from a terminal triacetyl arabinosyl residue and characterize the spectra of the monoterpene arabinoglucosides already isolated from these acetylated fractions of the grape [2]. Therefore, it was concluded that the longer R_t derivative was 2-phenylethyl hexa-*O*-acetyl 6-*O*-α-L-arabinofuranosyl-β-D-glucopyranoside (**1g**). Similarly, the first eluting 2-phenylethyl disaccharide acetate showed prominent ions at *m/z* 273, 153 and 111, characteristic of a terminal rhamnosyl triacetate residue as shown by analogous acetylated monoterpene β-rutinosides [2]. Accordingly, this compound was assigned as 2-phenylethyl hexa-*O*-acetyl β-rutinoside (**1e**).

Likewise, the accompanying pair of benzyl derivatives (**2e** and **2g**) showed, in addition to *m/z* 91 (tropylium ion) and *m/z* 152, 139, 110 and 97 (from the postulated triacetyl glucal fragmentation), the above spectral features of the two acetylated disaccharides.

GC of the TMSi derivatives of the glycosides **1d**, **1f**, **2d** and **2f** in the reversed phase fractions showed these as four peaks which came within the R_t range of the persilylated monoterpene disaccharides [2]. Also, as was observed with the monoterpene disaccharide analogues, the arabinoglucosides **1f** and **2f** were more abundant than the rutinosides **1d** and **2d**. While the chromatographic properties on the capillary column of the TMSi derivatives of **1d**, **1f**, **2d** and **2f** were superior to those observed for the acetates **1e**, **1g**, **2e** and **2g**, the EIMS of the TMSi derivatives gave less useful information about the sugar moieties than was obtained from the acetates, although the characteristic aglycone fragment ions were still clearly evident. In addition to disaccharide glycosides, TMSi derivatives of the glucopyranosides **1b** and **2b** were also present in these fractions. In TMSi samples of total reversed phase isolates from Muscat of Alexandria, the ratio of disaccharide glycosides (**1d**, **1f**, **2d**, **2f**) to glucosides (**1b**, **2b**) varied from *ca* 2:1 to 3:1, the monosaccharide derivatives being higher in concentration in samples from stored juice. It has previously been suggested [2] that the glucoside derivatives present in precursor fractions may arise by partial degradation of the labile 6-*O*-α-L-arabinofuranosyl-β-D-glucopyranosides.

Benzyl β-D-glucopyranoside (**2b**) has been found as a bitter principle in aqueous extracts of apricot kernels [8] and as a major neutral metabolite of benzyl alcohol in green-bug resistant barley [9]. A growth inhibiting active fraction of male flowers of *Cucurbita pepo* was also found to contain benzyl β-D-glucoside [10]. In addition, Russian workers have isolated 2-phenylethyl β-D-glucoside from rose flowers [11, 12], where it occurs, together with geranyl β-D-glucoside [13]. Both benzyl and 2-phenylethyl glycosides co-occur in the Stobbe's gland of nocuid moths [14].

The finding of the glycosides **1d**, **1f**, **2d** and **2f** in grapes appears to be the first observation of these aromatic alcohols in glycosidic combination with a sugar other than glucose. Furthermore, this work indicates that *Vitis vinifera* has the ability to form glycosides with rutinose and 6-*O*-α-L-arabinofuranosyl-β-D-glucose and a variety of alcohols of different biogenetic origin.

The glycosides **1b**, **1d**, **1f**, **2b**, **2d** and **2f** are precursors of the volatile alcohols **1a** and **2a** in grapes and will hydrolytically release the aromatic compounds into the juice. In *Vitis vinifera* species it can be assumed that **1a** and **2a** will contribute to the aromatic quality of the fruit along with the volatile monoterpene flavour compounds [15]. However, in other species, such as *Vitis labrusca*, where 2-phenylethanol (**1a**) is perceived to have an odour very reminiscent of labrusca character [16], the presence of such glycosides (**1b**, **1d**, **1f**) would assume great significance to the odour quality of those grapes.

EXPERIMENTAL

General exptal conditions are as previously described [2] with the following exceptions. High resolution MS on acetylated glucoside (**2c**) was obtained at 70 eV by direct insertion on a probe. GC/MS were determined at 25 and 70 eV, and separations were made on a glass WCOT column of SP2100 (29 m × 0.24 mm). Injections (0.1–5 μl) were made in the split mode. The column was held at 180° for 1 min and then programmed at 1°/min to 275°. Injector temp. was 280° and He was used as carrier gas at 28 cm/sec. The MS was scanned from *m/z* 35 to 800 per sec and the output data was computer processed.

For prep. TLC of acetylated compounds on Si gel 60 (1 mm) additional solvent systems included Et_2O and Et_2O–hexane (2:1). Unacetylated precursor concentrates were fractionated by prep. TLC on Si gel 60 (1 mm) using *iso*-PrOH–EtOAc–H_2O (6:3:1) as solvent.

Precursor fractions. Precursor concentrates were isolated by chromatography of various samples of juices from *Vitis vinifera* vars. Muscat of Alexandria and Rhine Riesling on a C_{18}-reversed phase adsorbent [1]. The material was eluted from the adsorbent directly with MeOH and not further fractionated for class separation of glycosidic precursors [1]. However, some of the precursor concentrates were rechromatographed on a C_8-reversed phase column prior to prep. TLC.

Synthesis of 2,3,4,6-tetra-O-acetyl β-D-glucopyranosides. 2,3,4,6-Tetra-O-acetyl α-D-glucopyranosyl bromide (4.1 g), Ag_2CO_3 (2.8 g) and either benzyl alcohol (2.16 g) or 2-phenylethanol (2.5 g) were stirred in dry Et_2O (50 ml) in the dark at room temp. for 7 days. The mixture was filtered and concd *in vacuo*. The crude product was purified by flash chromatography on Si gel 60, 230–400 mesh [17] with PhMe–Me_2CO (90:10) as solvent. Yields of pure materials were 16% for the benzyl derivative (**2c**) and 9% for the 2-phenylethyl derivative (**1c**).

Benzyl 2,3,4,6-tetra-O-acetyl β-D-glucopyranoside (**2c**). Colourless crystals from EtOH, mp 99–100° (cf. ref. [18] mp 100°), EIMS 25 eV, *m/z* (rel. int.): 347 (3), 331 (7), 259 (17), 245 (35), 216 (13), 169 (21), 157 (26), 152 (69), 139 (100), 110 (24), 97 (31), 91 (61), 43 (71).

The ^{13}C NMR spectrum (broad band decoupled, 20.1 MHz, $CDCl_3$) was in close agreement with that published [19].

2-Phenylethyl 2,3,4,6-tetra-O-acetyl β-D-glucopyranoside (**1c**). Colourless oil EIMS 25 eV, *m/z* (rel. int.): 347 (7), 331 (4), 169 (19), 157 (15), 145 (12), 112 (11), 109 (9), 105 (53), 104 (100), 98 (12), 81 (16), 43 (22). ^{13}C NMR spectrum (broad band decoupled, 20.1 MHz, $CDCl_3$, Me_4Si), δ100.8, 71.4, 72.9, 68.7, 72.0, 62.0 (glucose C-1–C-6, respectively), δ70.6, 36.0 (2-phenylethyl C-1 and C-2, respectively), δ138.6, 129.0, 128.4, 126.4 (phenyl C-1, C-2 and C-6, C-3 and C-5, C-4, respectively), δ20.5 (MeCO), 169.4–170.6 (MeCO). Assignments were based on published data [19, 20].

GC/MS of acetylated glycosidic precursors. A prep. TLC

fraction (R_f 0.6) from a reversed phase concentrate was acetylated and then analysed by GC/MS. This material showed peaks with MS fragmentation patterns corresponding to benzyl derivatives eluting at 37.5, 95.0 and 96.5 min and 2-phenylethyl derivatives eluting at 43.5, 97.75 and 103.0 min. The shortest R_t benzyl and 2-phenylethyl compounds gave spectra identical to those of synthetic 2,3,4,6-tetra-*O*-acetyl β-D-glucopyranosides of benzyl alcohol (**2c**) and 2-phenylethanol (**1c**). Additionally, these components in the acetylated precursor fraction were symmetrically peak enhanced on cochromatography with synthetic **1c** and **2c**. The peak at R_t 95.0 min, assigned as benzyl hexa-*O*-acetyl β-rutinoside (**2e**) showed at 25 eV, *m/z* (rel. int.): 41 (3), 42 (1), 44 (4), 57 (3), 71 (4), 91 (69), 96 (1), 97 (4), 110 (9), 111 (21), 127 (16), 139 (100), 140 (19), 143 (3), 152 (16), 153 (48), 157 (14), 170 (4), 207 (5), 273 (50).

The compound eluting at 96.5 min was assigned as benzyl hexa-*O*-acetyl 6-*O*-α-L-arabinofuranosyl-β-D-glucopyranoside (**2g**) and showed at 25 eV, *m/z* (rel. int.): 42 (3), 43 (16), 44 (1), 60 (2), 81 (1), 82 (1), 85 (1), 91 (15), 96 (2), 97 (3), 98 (1), 109 (3), 110 (4), 111 (1), 127 (6), 128 (3), 129 (3), 139 (73), 140 (9), 145 (1), 152 (8), 153 (4), 155 (2), 157 (3), 169 (14), 170 (4), 217 (1), 259 (100), 260 (9), 317 (6), 331 (4).

2-phenylethyl hexa-*O*-acetyl β-rutinoside (**1e**) was assigned as the peak emerging at 97.75 min and this showed at 25 eV, *m/z* (rel. int.): 42 (5), 43 (4), 60 (4), 68 (1), 69 (1), 81 (4), 82 (3), 96 (5), 104 (69), 105 (26), 110 (2), 111 (11), 127 (4), 128 (8), 140 (5), 152 (4), 153 (61), 154 (5), 157 (13), 170 (14), 171 (3), 207 (2), 273 (100), 317 (4).

The peak emerging at 103 min was assigned as 2-phenylethyl hexa-*O*-acetyl 6-*O*-α-L-arabinofuranosyl-β-D-glucopyranoside (**1g**) and showed at 25 eV, *m/z* (rel. int.): 42 (1), 43 (11), 44 (2), 45 (2), 57 (1), 60 (2), 69 (3), 81 (6), 96 (3), 97 (3), 98 (2), 104 (28), 105 (11), 109 (3), 110 (1), 136 (1), 139 (31), 153 (3), 157 (12), 169 (3), 170 (3), 207 (1), 221 (3), 259 (100), 260 (8), 281 (2), 355 (4), 429 (1).

Enzyme experiments. These were undertaken with Rohapect C as previously described [2] with the following modifications. Phthalate buffer at pH 5 was used for incubations. Standard solns of substrates were prepared by dissolving samples of precursor concentrates in pH 5 buffer and then liquid–liquid extracting these solns with Freon Fll for 72 hr to remove non-glycosidically bound material.

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