Phytochemistry, Vol. 21, No. 8, pp. 2013–2020, 1982.
Printed in Great Britain.

0031-9422/82/082013-08$03.00/0

NOVEL MONOTERPENE DISACCHARIDE GLYCOSIDES OF *VITIS VINIFERA* GRAPES AND WINES

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

(*Received* 23 *November* 1981)

Key Word Index—*Vitis vinifera*; Muscat of Alexandria; Rhine Riesling; grapes; wine; monoterpene glycoside; structure; β-rutinoside; 6-*O*-α-L-arabinofuranosyl-β-D-glucopyranosides; disaccharide.

Abstract—β-Rutinosides (6-*O*-α-L-rhamnopyranosyl-β-D-glucopyranosides) and 6-*O*-α-L-arabinofuranosyl-β-D-glucopyranosides of geraniol, nerol and linalol have been isolated and characterized in Muscat of Alexandria grapes and wine. These monoterpene disaccharide glycosides, which are precursors of linalol oxidation state monoterpenes of the grape, are also present in another non-muscat *Vitis vinifera* var. Rhine Riesling.

INTRODUCTION

Recent experiments [1] have demonstrated the presence of two forms of monoterpenoids in muscat grapes: (a) those free in the juice; and (b) non-volatile precursor forms. The non-volatile precursors give rise to members of the group (a) monoterpenes by hydrolytic reactions which proceed via a series of complex equilibria and may involve several intermediates. The recently identified hydroxylated linalol derivatives of muscat grapes [2] provide examples of such intermediates [1]. However, a full understanding of the relationship between the group (a) monoterpenes and the group (b) monoterpene derivatives depends on structural elucidation of the latter.

Cordonnier and Bayonove [3] first suggested that fruit of *Vitis vinifera* var. Muscat of Alexandria contained monoterpene glycosides of a sugar other than glucose. We have now isolated from grapes and wines of this variety, glycosidic derivatives which are precursors of linalol oxidation state monoterpenes [4]. In addition, the presence of these same compounds in at least one other *Vitis vinifera* var. Rhine Riesling is established. Structural studies on these glycosides are reported here.

RESULTS AND DISCUSSION

Precursor material was isolated from Muscat of Alexandria grape juice and wines by chromatography on a C_{18}-reversed phase absorbent as previously described [4]. The isolated material was assayed for monoterpenes by hydrolysis at pH 3 and GC analysis of the products. This procedure confirmed that the material under study gave predominantly linalol oxidation state monoterpenes [4].

Early experiments with precursor material involved electrophoresis in morpholine acetate buffer at pH 5, borate buffer at pH 9.2, and 0.1 M sodium hydroxide solution. Mobilities of the active material in these systems demonstrated that the natural product was not charged or readily ionized, thus precluding phosphate ester or protein groups, but showed it had properties consistent with a glycoside.

Trimethylsilylation of the precursor material followed by GC/MS indicated four major and several minor constituents. The two most abundant products showed fragment ions on EIMS at *m/z* 191 ($C_7H_{19}Si_2O_2$), 204 ($C_8H_{20}Si_2O_2$) and 217 ($C_9H_{21}Si_2O_2$), diagnostic of a trimethylsilylated carbohydrate [5]. Additionally, prominent ions at *m/z* 69 (C_5H_9) and 81 (C_6H_9) were attributed to hydrocarbon fragments from the monoterpene portion of the natural material. The GC RR_t's of the trimethylsilylated precursors were considerably longer than those of similarly derivatized synthetic β-D-glucopyranosides of monoterpene alcohols. These data suggested that the grape monoterpene precursor material consisted of several glycosidic derivatives in which the sugar moiety was larger than a monosaccharide.

Hydrolytics studies were then undertaken in order to investigate both the glycosidic and monoterpene parts of the natural product. These included acid catalysed hydrolyses at pH 1 and 3, both before and after hydrogenation, of the isolated material. Similarly, products formed on enzymatic hydrolysis were investigated.

Mild acid treatment at pH 3.0 led to only partial hydrolysis and the carbohydrate fragments were investigated by GC/MS of the TMSi derivatives. Thus the presence of arabinose and glucose was established in the hydrolysate. Also two other major and several minor trimethylsilylated sugars were detected. From GC studies with TMSi derivatives of reference mono-, di- and trisaccharides, the RR_t's of the unknown compounds suggested that they were disaccharide derivatives.

When more vigorous hydrolytic conditions, at pH 1, were applied to the natural material, GC analysis of the TMSi hydrolysis products then showed that disaccharides were largely absent. However, the monosaccharides now included rhamnose together with arabinose and glucose.

Another minor but significant product identified in the pH 3 hydrolysis of the natural precursor was geranyl-β-D-glucopyranoside (**1a**). This observation was confirmed when similar GC analyses of products from the pH 3 hydrolysis of the hydrogenated natural material demonstrated substantial quantities of 3, 7-dimethyloct-1-yl-β-D-glucopyranoside.

These results implied that the natural precursor material was a mixture of disaccharide glycosides made up of glucose, arabinose and rhamnose. The identification of geranyl-β-D-glucopyranoside (**1a**) in the partial hydrolysate indicated that the terpene residue was bound to the disaccharides through a glucopyranose.

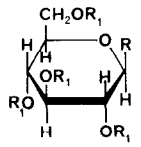
Investigations of the aglycones were made by GC/MS analyses of the monoterpenes formed on hydrolysis of the natural material. At pH 3 it gave predominantly the alcohols linalol, α-terpineol, geraniol and nerol. Minor amounts of monoterpene hydrocarbons were also produced. Subsequent hydrolysis experiments with synthetic glucosides of monoterpene alcohols have shown that rearrangements can occur and terpenic products may not be characteristic of the substrate [Williams, P. J., Strauss, C. R., Wilson, B. and Massy-Westropp, R. A., unpublished]. To prevent rearrangements of the aglycones the precursor material was hydrogenated and then hydrolysed at pH 3. This procedure gave the saturated tertiary alcohols, 3,7-dimethyloctan-3-ol, *cis*- and *trans*-2-(4-methylcyclohexyl)propan-2-ol. 3, 7-Dimethyloctan-1-ol was not produced until prolonged hydrolysis at pH 1 was carried out. These results established that the grape terpene precursor material was made up of aglycones of three different structural types, corresponding to linalol, α-terpineol and nerol–geraniol.

To identify the aglycone monoterpenes, and to determine the relative ratios of the various terpene alcohols present, a series of enzyme catalysed hydrolyses was next undertaken. The enzymes employed were almond β-glucosidase (emulsin) EC 3.2.1.21, and a commercial pectinase preparation, Rohapect C, which was demonstrated to possess potent glycosidase activity with geranyl β-D-glucopyranose (**1a**). The emulsin showed only weak activity towards the natural grape precursors, consistent with earlier observations of Cordonnier and Bayonove [3]. On the other hand, Rohapect C, under similar conditions, exhibited a 10-fold activity over emulsin and appeared to completely hydrolyse the substrates. The monoterpenes produced by both enzymes were geraniol, nerol and linalol and in the case of Rohapect C they were liberated in a ratio of 3 : 1 : 2. Whilst trace quantities of α-terpineol and citronellol were also observed with the latter enzyme, no other monoterpenoids were detected.

The data thus far indicated that the monoterpene glycosides isolated from muscat grape juice were a complex mixture in which three different aglycones predominated and at least two different disaccharide moieties were involved. Work was next directed towards the elucidation of some specific components of this mixture.

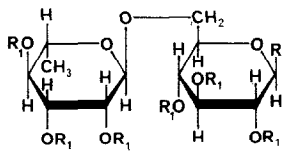
Precursor material was acetylated and LC carried out on Si gel. Two fractions were isolated and these were shown by deacetylation, hydrolysis and GC analysis, to contain all the monoterpene yielding material of the natural product.

The higher R_f, minor acetate fraction had the following significant fragment ions on EIMS: *m/z* 561 ($C_{24}H_{33}O_{15}$ rhamnoglucosyl hexa-acetate) and 273 ($C_{12}H_{17}O_7$ rhamnosyl triacetate). The latter fragment suggested that the rhamnosyl residue was terminal in this disaccharide. ^{1}H NMR spectroscopy of the material showed signals consistent with those expected for rhamnoglucosyl disaccharides of the monoterpene alcohol mixture already established. The ratio of signals centred at δ 3.6 and 4.2 provided the key to the interpretation of this spectrum. These signals, for protons on carbon bearing non-acetylated oxygen and for protons on carbohydrate methylenes carrying acetoxy groups respectively, have been delineated by Binkley *et al.* [6] in polysaccharide structural analyses. Under the signal at δ 4.2 in the higher R_f acetate fraction, the allylic methylene protons at C-1 of the geranyl and neryl residues also appear. From the known proportion of nerol and geraniol present in the aglycone mixture it was thus possible to assign the signal at δ 4.2 to these allylic protons. Accordingly, the protons on C-6 of the glucose unit were not under



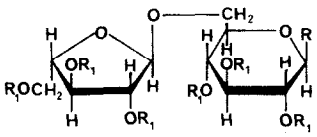
1 R_1 = H

2 R_1 = Ac



3 R_1 = H, Rutinoside (6-*O*-α-L-rhamnopyranosyl-β-D-glucopyranoside)

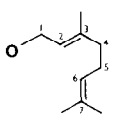
4 R_1 = Ac



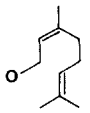
5 R_1 = H, 6-*O*-α-L-arabinofuranosyl-β-D-glucopyranoside

6 R_1 = Ac

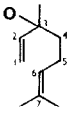
a R =



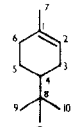
b R =



c R =



d R =



e R = OH

f R = OAc

the envelope at δ 4.2 and hence the oxygen on C-6 of the glucose was not acetylated. Therefore, the disaccharide moiety was a rhamnosyl-6-*O*-β-D-glucopyranoside.

These deductions were confirmed by comparison of the chromatographic properties of the disaccharides from the natural precursors with rutinose (6-*O*-α-L-rhamnopyranosyl-β-D-glucopyranose) (**3e**) [7]. This sugar was obtained by controlled acid hydrolysis [8] of the flavonoid rutin. Authentic rutinose TMSi gave two peaks on GC. These TMSi rutinose epimers cochromatographed with the TMSi derivatives of the two major disaccharides obtained on hydrolysis of the precursor at pH 3.

Synthesis of authentic geranyl- and neryl-β-rutinoside hexa-acetates (**4a**, **4b**) under Köenigs–Knorr conditions provided reference compounds and ^{13}C NMR spectroscopy established the stereochemistry of their glycosidic linkages. Comparison of the ^{1}H NMR, mass spectral, TLC and GC/MS properties of these authentic materials confirmed them as major constituents of the higher R_f fraction of the acetylated natural product.

EIMS of the lower R_f acetate fraction showed ions at *m/z* 547 ($C_{23}H_{31}O_{15}$, arabinoglucosyl hexa-acetate) and 259 ($C_{11}H_{15}O_7$, arabinosyl triacetate). By analogy with the rutinoside fragmentation these ions indicated that the disaccharide moiety in this major acetate fraction was an arabinoglucoside.

The 80 MHz ^{1}H NMR spectrum of the major acetate fraction was too complex to allow a detailed analysis of the structure of the arabinoglucoside. However, integration of signals at δ 3.7 and 4.2 in the spectrum showed approximately equal numbers of protons under these two envelopes. Interpretation of these data in a similar manner to that employed on the rutinoside spectrum indicated that the disaccharide moiety was either a 6-*O*-arabinofuranosyl-D-glucopyranose or a 2-, 3- or 4-*O*-arabinopyranosyl-D-glucopyranose.

Broad band decoupled ^{13}C NMR spectra were recorded on the major acetate fraction as well as on the deacetylated product. This latter determination was made because the available ^{13}C NMR spectral data for acetylated carbohydrates are not as comprehensive as those for free sugars. Interpretation of the spectrum of the acetylated material was facilitated by comparison with the ^{13}C NMR spectra of the synthetic 2, 3, 4, 6-tetra-*O*-acetyl-β-D-glucopyranosides of geraniol, nerol and linalol (**2a**, **2b**, **2c**) and also with the methyl 2,3,5-tri-*O*-acetyl arabinofuranoside isomers (see Table 1).

These spectra indicated that the natural product was a mixture containing predominantly geranyl, neryl and linalyl aglycones. However, peaks for the glycosidic carbons in the ^{13}C NMR spectra of the natural material, suggested that a single disaccharide moiety was present. Thus β-D-glucopyranoside C-1 carbons at δ 99.1, 98.6 and 95.9, in the acetylated natural product were assigned to those bearing neryl, geranyl and linalyl residues, respectively. One other anomeric carbon, which appeared at a particularly low field (δ 109) in the spectrum of the deacetylated fraction, was strongly indicative of an α-arabinofuranosyl unit in the natural disaccharide [9]. Accordingly, this fraction of the precursor material from the grapes appeared to be a mixture of monoterpene 6-*O*-α-arabinofuranosyl-β-D-glucopyranosides (**5a**, **5b**, **5c**). Two features of the ^{13}C NMR spectrum of the acetylated natural product gave further support to this disaccharide structure. Firstly, the absence of a signal for the C-6 of the glucose bearing acetate [10], which had appeared at δ 62.1–62.5 in spectra of the three model acetylated monoterpene glucosides (Table 1), was consistent with an intersugar link in the disaccharide through C-6 of glucose. Secondly, signals at δ 63.3, 77.0, 80.2, 81.1 and 105.9 closely corresponded with those for the pentose carbons in the spectrum of reference α-methyl 2,3,5-tri-*O*-acetyl arabinofuranoside. The deshielding of the last three of these signals was particularly diagnostic since they appeared at much lower field than comparable signals in β-methyl 2, 3, 5-tri-*O*-acetylarabinofuranoside.

At this point it was necessary to determine the absolute configuration of the arabinose unit of the disaccharide. The method of Gerwig *et al.* [11], which involved GC/MS analysis of the diastereoisomeric glycosides formed with optically active 2-butanol, was appropriate to the micro-scale required for this investigation. Application of this technique to the arabinose obtained on acid hydrolysis of the major precursor demonstrated that the pentose had the L-configuration.

Finally the disaccharide structure of this major monoterpene precursor was confirmed by comparison of the NMR spectral properties of the acetylated product with those of an authentic sample of hepta-*O*-acetyl-6-*O*-α-L-arabinofuranosyl-β-D-glucose (**6f**) [12]. Whilst the ^{1}H NMR spectra of these two showed signal multiplicities having close similarity, the carbon spectra gave unequivocal confirmation of the identity (Table 1). Thus, 9 of the 11 carbohydrate carbon signals corresponded in the spectra of the natural product and reference compound (**6f**). Only the signals for glucose C-1 and C-2 in the spectrum of (**6f**) differed markedly from those of the precursor disaccharide, owing to the substitution of an acetoxy group rather than a monoterpene aglycone on the glucose C-1.

With the structures of six Muscat of Alexandria glycosides determined as (**3a–3c** and **5a–5c**) another grape variety was investigated for the presence of these compounds. It is of interest to examine several cultivars for precursors as these compounds may have significance to varietal character. Whilst a comprehensive survey is beyond the scope of the present work, it has been shown that these same six glycosides are present in Rhine Riesling grape juice. Thus, by cochromatography of acetylated precursor fractions from Muscat of Alexandria and Rhine Riesling, using TLC, GC and GC/MS it was confirmed that (**3a–3c** and **5a–5c**) are constituents of this second *Vitis vinifera* variety. Notably, however, Rhine Riesling appeared to contain less of these glycosides than the *ca* 1.5 ppm (minimum, based on isolated material) observed in Muscat of Alexandria juice although a relatively greater proportion of linalyl glycosides (**3c** and **5c**) was seen in the former variety.

The discovery of these glycosides (**3a–3c** and **5a–5c**) in *Vitis vinifera* grapes appears to be the first instance of disaccharides occurring in glycosidic

Table 1. ^{13}C

	Chemical shift				
Carbon	α-MTA*	β-MTA*	**6f**†	**2a**†	**2b**†
CH_3-O	55.0	55.5	—	—	—
A1§	107.0	101.4	106.2	—	—
A2‖	80.4	77.0	80.4	—	—
A3‖	77.4	76.0	77.0	—	—
A4‖	81.5	78.8	81.1	—	—
A5	63.4	65.5	63.2	—	—
G1§	—	—	91.7	98.7	99.0
G2¶	—	—	70.3	71.3 or 71.8	71.4 or 71.8
G3¶	—	—	72.9	73.0	73.0
G4¶	—	—	68.6	68.6	68.6
G5¶	—	—	74.0	71.8 or 71.3	71.8 or 71.4
G6¶	—	—	66.0	62.1	62.1
R1§	—	—	—	—	—
R2††	—	—	—	—	—
R3††	—	—	—	—	—
R4††	—	—	—	—	—
R5††	—	—	—	—	—
R6††	—	—	—	—	—
M1§,**	—	—	—	65.1	65.1
M2**	—	—	—	119.3	120.4
M3**	—	—	—	142.1	142.0
$\underline{C}H_3$-M3**	—	—	—	16.0	23.3
M4**	—	—	—	39.3	31.9
M5**	—	—	—	26.1	26.5
M6**	—	—	—	123.8	123.6
M7**	—	—	—	131.8	132.1
trans $\underline{C}H_3$-M7**	—	—	—	25.4	25.4
cis $\underline{C}H_3$-M7**	—	—	—	17.5	17.5
$\underline{C}H_3COO$	20.8	20.8	20.6	20.4	20.4
$CH_3\underline{C}OO$	169.8, 170.3, 170.8	170.5–170.8	169.0–170.7	169.2–170.5	169.3–170.5

*α- and β-MTA = α- and β-methyl-2, 3, 5-tri-*O*-acetyl-L-arabinofuranosides.
†Synthetic products.
‡Lower R_f (0.39) acetylated natural product.
§A = L-arabinose; G = D-glucose; R = L-rhamnose; M = monoterpene.
‖Assigned from data for unacetylated arabinosides [9].
¶Assigned from published data [10] in analogous carbohydrates.
**Monoterpene carbon shifts assigned from data of ref. [26].
††Rhamnose carbons assigned from data of ref. [27].
‡‡Signal at *ca* δ 131.8 not observed.

NMR spectral data of each compound

2c†	4a†	4b†	6a‡	6b‡	6c‡
—	—	—	—	—	—
—	—	—	105.9	105.9	105.9
—	—	—	80.2	80.2	80.2
—	—	—	77.0	77.0	77.0
—	—	—	81.1	81.1	81.1
—	—	—	63.3	63.3	63.3
95.9 and 96.2	98.8	99.2	98.6	99.1	95.9
71.6	71.0	71.4	71.4	71.4	71.4
73.2	73.0	73.0	73.1	73.1	73.1
69.0	68.9	68.9	69.2	69.2	69.2
71.6	73.3	73.0	73.1	73.1	73.1
62.5	66.6	66.6	66.1	66.1	66.1
—	98.2	98.1	—	—	—
—	70.2	69.5	—	—	—
—	68.7	69.5	—	—	—
—	70.9	71.1	—	—	—
—	67.3	67.0	—	—	—
—	17.5	17.2	—	—	—
114.2	65.2	65.3	65.2	65.2	114.3
141.9 and 142.5	119.3	120.4	119.3	120.4	142.1
80.8	142.5	142.1	142.3	142.1	80.4
23.1	16.2	23.3	16.3	23.4	23.1
40.3 and 41.7	39.5	31.9	39.1	31.3	41.6
22.2	26.2	26.5	26.0	26.2	22.2
124.3	122.3	123.8	123.8	123.8	124.0
131.8	131.8	‡‡	131.6	131.6	131.6
25.5	25.5	25.5	25.5	25.5	25.5
17.5	17.3	17.2	17.6	17.6	17.6
20.5	20.4 and 20.6	20.5	20.5 and 20.7	20.5 and 20.7	20.5 and 20.7
169.2–170.7	169.4–170.4	169.4–170.4	168.8–170.2	168.8–170.2	168.8–170.2

combination with monoterpenoids. Whilst rutinose (**3e**) is a well-known sugar occurring in flavonoid [13] and phenolic [14] glycosides, 6-*O*-α-L-arabinofuranosyl-β-D-glucopyranose (**5e**) was hitherto unknown as a natural compound. The lability of the inter-sugar link in (**5e**), due to the arabinofuranosyl residue, [15] should alert workers in this field to the possibility of the more widespread occurrence of this sugar in nature. Thus, glycosides of (**5e**) could escape detection in natural products owing to degradation during isolation and work-up, leaving a β-D-glucoside. Indeed the presence of small and varying quantities of β-D-glucosides (**1a–1c**) observed in the grape precursor fractions may well be accounted for by such a partial degradation of (**5a–5c**). These disaccharide glycosides of the monoterpenes (**3a–3c** and **5a–5c**) whilst showing sensitivity to mild acid hydrolysis similar to that of the model glucoside derivatives (**1a–1c**) [Williams, P. J., Strauss, C. R., Wilson, B. and Massy-Westropp, R. A., unpublished] are much more stable to common plant β-glucosidases, e.g. emulsin. Accordingly, specific enzymes would need to be present in the grape in order to cleave the natural glycosides and so liberate the free monoterpenes of the fruit by a largely enzymatic route. The original work of Cordonnier and Bayonove [3] suggested that such enzyme systems may exist in Muscat of Alexandria. However, the isolation of significant quantities of monoterpene glycosidic precursors from finished wines indicates that such specific glycosidases are not very active during must processing and fermentation.

A simple acid hydrolytic route is considered to be a major pathway to the free monoterpenes of grapes because of the abundance of α-terpineol found in muscat grape juices [16, 17]. α-Terpineol has only a trace glycosidic precursor in the fruit, but it is a major end product of acid catalysed hydrolyses of geranyl, neryl and linalyl glycosides [Williams, P. J., Stauss, C. R., Wilson, B. and Massy-Westropp, R. A., unpublished].

EXPERIMENTAL

MS were determined at 20–30 eV by direct insertion on a probe or at 70 eV by GC/MS. ^{1}H NMR microprobe spectra were obtained at 80 MHz with Fourier transform, in $CDCl_3$ and with TMS or $CHCl_3$ as int. standard. ^{13}C NMR (20.1 MHz) broad band decoupled, microprobe spectra were run in $CDCl_3$ with TMS or $CDCl_3$ as int. standard. Spectra of deacetylated products were obtained in D_2O with *t*-BuOH as int. standard.

TMSi derivatives were prepared with HMDS–TMCS–pyridine (2 : 1 : 7) as reagent. Hydrogenations were carried out with PtO_2 as catalyst in MeOH at atm pres. For TLC of acetylated compounds Si gel 60 with PhMe–Me_2CO (4 : 1) as developing solvent and anisaldehyde–HOAc–H_2SO_4 (1 : 100 : 2) as spray reagent were used. TLC of deacetylated products also utilized Si gel 60, with EtOH–EtOAC (3 : 17) or *n*-PrOH–EtOAc–H_2O (3 : 1 : 1) as solvents and the above spray reagent. Mps are uncorr.

GC and GC/MS were carried out under several sets of conditions. To chromatograph volatile monoterpenes. (a) A glass SCOT column (96 m × 0.5 mm) of SP1000 on Chromosorb R was used. The column was operated isothermally at 50° for 10 min then temp. programmed to 180° at 1°/min and held at 180° for 20 min. Injector and FID temps. were 225°. Carrier gas was N_2 at 2.8 ml/min flow rate, with septum purge of 150 ml/min. (b) A glass WCOT column (40 m × 0.2 mm) coated with Carbowax 20M was used for GC/MS analyses. Injections were made with a 12 : 1 split. The column was held at 60° for 10 min and then programmed at 1°/min to 160° and held at the upper temp. for 30 min. He carrier gas head pres. was 55 kPa. The injector and glass transfer line temps were 250°. Spectra were taken each second, scanning from *m/z* 35 to 450.

For GC and GC/MS of TMSi derivatives, viz. monoterpene precursor substances, carbohydrate residues from hydrolyses and model compounds, the samples were silylated after desiccation *in vacuo* over KOH at room temp. Analytical GC was performed under the following conditions. (c) On a glass SCOT column (61 m × 0.5 mm) coated with SE30 and operated from 120 to 300° at 2°/min, then held at 300°. N_2 carrier gas flow rate was 3.4 ml/min and injector and FID temps. were 250°. GC/MS studies of the above TMSi derivatives were made under the following conditions. (d) The same SE30 column was employed with He as carrier gas at head pres. 135 kPa. The oven was programmed from 120 to 290° at 2°/min and held at 290° for 5 min. Injections of 2–3 μl were made with a 10 : 1 injection split. Injector temp. was 300° and detection by total ion monitor, scanning at 70 eV from *m/z* 35 to 800 per sec. (e) TMSi monosaccharides were analysed on an aged Carbowax 20M glass column [18] which was programmed from 120 to 170° at 1°/min. Other operating parameters were the same as those in (a) above. (f) TMSi (+)2-butyl arabinoside diastereoisomers were resolved on a glass WCOT column (30 m × 0.24 mm) of SP2100. Injections (0.2–2 μl) were made by the Grob method. The column was held at 70° for 2 min, and 0.5 min after injection, the split (20 : 1) was opened. The column was programmed at 10°/min to 120° and thence at 1°/min to 250°. Injector temp. was 250° and detection was by mass spectrometer. (g) Acetylated precursor fractions were subjected to GC/MS using a fused Si WCOT column (3 m × 0.2 mm) coated with OV101. Grob-type injections were made, and samples (0.2–0.5 μl) were injected at a column temp. of 60°. After 2 min the column was programmed at 10°/min to 180° and then at 3°/min to 280°. The injector was vented 0.1 min after injection. Injector temp. was 280° and detection was by mass spectrometer.

Isolation of precursor substances. The juice of *V. vinifera* grapes, var. Muscat of Alexandria (syn. Muscat Gordo Blanco) (15.75 l.) and var. Rhine Riesling (1.7 l.) were chromatographed on a C_{18}-reversed phase adsorbent as previously described [4]. From Muscat of Alexandria, a fraction which yielded predominantly linalol oxidation state monoterpenes on hydrolysis, was desiccated to give a residue of 100 mg. This material was used for structural studies.

A portion of the above residue (*ca* 90 mg) was acetylated and then fractionated by LC on Si gel 60. Two major fractions were obtained—the higher R_f minor fraction (6 mg), R_f 0.44 and the lower R_f major fraction (27 mg), R_f 0.39.

Spectral data for the higher R_f *fraction.* EIMS (probe) 30 eV, *m/z* (rel. int.): 561 (0.5), 331 (3), 273 (81), 213 (20), 171 (22), 169 (21), 153 (100), 139 (30), 137 (43), 136 (91), 121 (24), 111 (64), 109 (21), 94 (16), 93 (74), 92 (32), 83 (18), 81 (66), 80 (69), 69 (43), 43 (90). ^{1}H NMR (80 MHz, $CDCl_3$): assignments based on geranyl–neryl–linalyl derivative ratio of 1.5 : 0.5 : 1; (150H total); 1.17 (9H, *d*, rhamnose C-6), 1.27 (3H, *s*, Me–linalyl C-3), 1.58 (24H, *br d*, Me–geranyl, neryl and linalyl C-7 and Me–geranyl and neryl C-3), 2.09 (66H, *m*, Me's of rutinose acetates, H on geranyl, neryl and linalyl

C-4 and C-5), 3.64 (12H, *br m*, H on glucose C-5 and C-6, H on rhamnose C-5), 4.20 (4H, *d*, H on neryl and geranyl C-1), 4.5 (3H, *m*, H on glucose C-1), 4.7–6.0 (29H, *m*, H on glucose C-2, C-3 and C-4, H on rhamnose C-1, C-2, C-3 and C-4, H on geranyl and neryl C-2 and C-6, H on linalyl C-1, C-2 and C-6). There was an impurity in the sample which was reduced in proportion but not completely eliminated by repeated chromatography on Si gel 60. This material gave high field signals at δ 1.17 and 1.27.

Spectral data for the lower R_f *fraction.* EIMS (probe 25 eV, *m/z* (rel. int.): 547 (0.4), 317 (1.7), 259 (99), 139 (100), 137 (29), 136 (67), 121 (13), 97 (22), 93 (53), 92 (25), 81 (56), 80 (61), 69 (36), 43 (70). ^{1}H NMR (80 MHz, $CDCl_3$): assignments based on geranyl–neryl–linalyl derivative ratio of 1.5 : 0.5 : 1. Diagnostic signals at 3.66 (12H, *m*, H on glucose C-5 and C-6, H on arabinofuranose C-4), 4.23 (10H, *m*, H on arabinofuranose C-5, H on geranyl and neryl C-1). Other signals were observed at 1.28 (Me–linalyl C-3), 1.6 (Me–geranyl, neryl and linalyl C-7 and Me–geranyl and neryl C-3), 2.07 (Me's from arabinoglucose acetates, H on geranyl, neryl and linalyl C-4 and C-5), 4.56 (H on glucose C-1), 4.7–6.0 (H on glucose C-2, C-3 and C-4, H on arabinofuranose C-1, C-2 and C-3, H on geranyl and neryl C-2 and C-6, H on linalyl C-1, C-2 and C-6). There was an impurity in the sample which was reduced in proportion but not completely eliminated by repeated chromatography on Si gel 60. This material showed signals at δ 0.9 and 1.28. For ^{13}C NMR spectrum see Table 1.

Synthesis of 2,3,4,6-*tetra*-O-*acetyl* β-D-*glucoside derivatives of monoterpene alcohols.* Ag_2O, freshly prepared [19], was dried in the dark for 3 days before use. Tetra-*O*-acetyl-α-D-glucopyranosyl bromide (acetobromoglucose) mp 86–88° was prepared according to ref. [20]. Tetra-*O*-acetyl β-D-glucopyranosides of monoterpenes (**2a–2d**) were synthesized under the following general Koenigs–Knorr conditions. Acetobromoglucose (4.5 g), monoterpene alcohol (11.2 g) and dried Ag_2O (3.0 g) were stirred in dry Et_2O (100 ml) in the dark at room temp. for 12 days. The mixture was filtered, concd *in vacuo* and excess monoterpene alcohol removed by steam distillation. The crude product was extracted with Et_2O and purified by LC on Si gel 60 with PhMe–Me_2CO as solvent. Yields of purified material ranged from 21% for the linalyl tetra-*O*-acetyl β-D-glucopyranoside (**2c**) to 65% for the geranyl derivative (**2a**).

Geranyl tetra-O-*acetyl* β-D-*glucopyranoside* (**2a**). Colourless oil, R_f 0.55. EIMS (probe) 20 eV, *m/z* (rel. int.): 332 (7), 331 (8), 271 (3), 211 (3), 169 (100), 137 (27), 136 (31), 137 (24), 136 (79), 123 (30), 121 (23), 110 (21), 109 (60), 97 (17), 94 (17), 93 (63), 92 (21), 81 (44), 80 (43), 69 (47), 68 (35), 43 (51). ^{1}H NMR (80 MHz, $CDCl_3$) 1.51 (3H, *s*, *transoid* Me–geranyl C-7), 1.57 (6H, *br s*, Me–geranyl C-3 and C-7), 1.94 (16H, 4*s* plus *m*, acetate Me's and H on geranyl C-4 and C-5), 3.6 (1H, *m*, H on glucose C-5), 4.16 (4H, *m*, H on geranyl C-1 and H on glucose C-6), 4.45 (1H, *d*, J = 7.5 Hz, H on glucose C-1), 4.7–5.5 (5H, *m*, H on glucose C-2, C-3 and C-4, H on geranyl C-2 and C-6). For ^{13}C NMR see Table 1.

Neryl tetra-O-*acetyl* β-D-*glucopyranoside* (**2b**). Colourless plates from aq. EtOH, mp 68–70°, R_f 0.55. EIMS (probe) 20 eV, *m/z* (rel. int.): 332 (5), 331 (32), 271 (7), 169 (100), 137 (25), 136 (44), 121 (16), 109 (37), 93 (55), 92 (25), 81 (38), 80 (53), 69 (17), 68 (25), 43 (79). ^{1}H NMR (80 MHz, $CDCl_3$) 1.50 (3H, *s*, *transoid* Me–neryl C-7), 1.58 (3H, *s*, *cisoid* Me–neryl C-7), 1.67 (3H, *s*, Me–neryl C-3) 1.94 (16H, 4*s*, plus *m*, acetate Me's and H on neryl C-4 and C-5), 3.59 (1H, *m*, H on glucose C-5), 4.14 (4H, 2*d*, H on neryl C-1 and H on glucose C-6), 4.45 (1H, *d*, J = 7.5 Hz, H on glucose C-1), 4.7–5.5 (5H, *m*, H on glucose C-2, C-3 and C-4, H on neryl C-2 and C-6).

α-Terpinyl tetra-O-*acetyl* β-D-*glucopyranoside* (**2d**). Colourless crystals from aq. EtOH, mp 106–108°, R_f 0.55. EIMS (probe) 20 eV, *m/z* (rel. int.): 331 (9), 322 (4), 271 (3), 259 (2), 245 (3), 169 (19), 137 (34), 136 (100), 121 (6), 109 (4), 93 (6), 92 (6), 81 (11). ^{1}H NMR (80 MHz, $CDCl_3$) 1.04 and 1.11 (6H, 2*s*, Me-α-terpinyl C-8), 1.56 (6H, *m*, Me-α-terpinyl C-1, H on α-terpinyl C-4 and C-5), 1.95 (16H, 4*s* plus *m*, acetate Me's plus H on α-terpinyl C-3 and C-6), 3.63 (1H, *m*, H on glucose C-5), 4.26 (2H, *m*, H on glucose C-6), 4.62 (1H, *d*, J = 7.5 Hz, H on glucose C-1), 4.75–5.42 (4H, *m*, H on glucose C-2, C-3 and C-4, H on α-terpinyl C-2).

(±)*Linalyl tetra*-O-*acetyl* β-D-*glucopyranoside* (**2c**). Colourless oil, R_f 0.55. EIMS (probe) 20 eV, *m/z* (rel. int.): 331 (6), 271 (2), 259 (2), 169 (38), 138 (18), 137 (32), 136 (69), 121 (17), 109 (24), 94 (20), 93 (88), 92 (37), 81 (48), 80 (100), 69 (18), 43 (27). ^{1}H NMR (80 MHz, $CDCl_3$) 1.19 and 1.27 (3H, 2*s*, Me–linalyl C-3), 1.53 (3H, *s*, *transoid* Me–linalyl C-7), 1.61 (3H, *s*, Me–linalyl C-7), 1.98 (16H, 4*s* plus *m*, acetate Me's and H on linalyl C-4 and C-5), 3.61 (1H, *m*, H on glucose C-5), 4.09 (2H, *m*, H on glucose C-6), 4.50 and 4.55 (1H, 2*d*, J for each = 7.5 Hz, H on glucose C-1), 4.77–5.42 (6H, *m*, H on glucose C-2, C-3 and C-4, H on linalyl C-1 and C-6), 5.78 (1H, *q*, H on linalyl C-2). For ^{13}C NMR see Table 1.

Synthesis of monoterpene hexa-O-*acetyl* β-*rutinosides. Quercetin-3-*β-*rutinoside* (rutin) (10.0 g) was hydrolysed by refluxing in 10% HOAc (1.8 l.) for 2 hr [8]. The aq. soln was concd to dryness, resuspended in H_2O (50 ml) and filtered. The filtrate was passed down a small column of C_{18}-reversed phase adsorbent and the total aq. eluate (60 ml) containing rutinose, glucose and rhamnose (total dry wt 3.2 g) acetylated. Hepta-*O*-acetyl-β-rutinoside (**4f**) (1.82 g) (colourless rods from aq. EtOH, mp 169–171° cf lit. [21] mp 169–170°) was isolated by LC on Si gel 60 using a PhMe–Me_2CO solvent gradient. The product (0.33 g) was converted to acetobromorutinose [22]* and this was taken up in dry Et_2O (15 ml) containing Ag_2CO_3 (0.7 g) and dry $MgSO_4$. Nerol or geraniol (1 g) was added and the mixture stirred in the dark at room temp. for 7 days. The mixture was filtered, concd *in vacuo* and the excess monoterpene alcohol removed by steam distillation. The crude product was extracted with $CHCl_3$ and subjected to LC on Si gel 60 (PhMe–Me_2CO solvent) to yield the product geranyl or neryl hexa-*O*-acetyl-β-D-rutinosides (10.2 or 10.8 mg respectively).

Geranyl hexa-O-*acetyl* β-*rutinoside* (**4a**). Colourless oil, R_f 0.44. EIMS (probe) 20 eV, *m/z* (rel. int.) 317 (5), 289 (2), 274 (11), 273 (78), 213 (21), 184 (15), 171 (20), 157 (15), 153 (94), 139 (33), 137 (29), 136 (100), 123 (16), 122 (20), 121 (19), 111 (45), 109 (11), 93 (63), 92 (22), 81 (32), 80 (32), 69 (30), 43 (41). ^{1}H NMR (80 MHz, $CDCl_3$) 1.15 (3H, *d*, J = 6.3 Hz, rhamnose C-6), 1.55 (3H, *s*, *transoid* Me–geranyl C-7), 1.59 (6H, *br s*, Me–geranyl C-3 and C-7), 2.0 (22H, *m*, acetate Me's plus H on geranyl C-4 and C-5), 3.69 (4H, *m*, H on glucose C-5 and C-6, H on rhamnose C-5), 4.16 (2H, *d*, J = 6.8 Hz, H on geranyl C-1), 4.47 (1H, *d*, J = 7.5 Hz, H on glucose C-1), 4.70–5.48 (9H, *m*, H on glucose C-2, C-3 and C-4, H on rhamnose C-1, C-2, C-3 and C-4, H on geranyl C-2 and C-6). For ^{13}C NMR see Table 1.

*These authors erroneously assigned the rutinose inter-sugar linkage as β.

Neryl hexa-O-acetyl β-rutinoside (**4b**). Colourless oil, R_f 0.44. EIMS (probe) 30 eV, *m/z* (rel. int.): 317 (2), 274 (4), 273 (40), 213 (9), 171 (15), 153 (66), 139 (11), 137 (26), 136 (33), 121 (12), 111 (54), 109 (10), 93 (45), 92 (16), 83 (15), 81 (62), 80 (34), 69 (34), 68 (18), 43 (100). ^{1}H NMR (80 MHz, $CDCl_3$) 1.22 (3H, *d*, $J = 6.3$ Hz, rhamnose C-6), 1.61 (3H, *s*, *transoid* Me-neryl C-7), 1.69 (3H, *s*, *cisoid* Me-neryl C-7), 1.77 (3H, *s*, Me-neryl C-3), 2.04 (22H, 4*s*, plus *m*, acetate Me's plus H on neryl C-4 and C-5), 3.75 (4H, *m*, H on glucose C-5 and C-6, H on rhamnose C-5), 4.23 (2H, *d*, $J = 7.5$ Hz, H on neryl C-1), 4.56 (1H, *d*, $J = 7.5$ Hz, H on glucose C-1), 4.75–5.50 (9H, *m*, H on glucose C-2, C-3 and C-4, H on rhamnose C-1, C-2, C-3 and C-4, H on neryl C-2 and C-6). For ^{13}C NMR spectrum see Table 1.

Synthesis and isolation of methyl L-*arabinosides.* The four methyl L-arabinoside isomers were prepared and then separated by ion exchange [23]. Optical rotations of the products [24] confirmed that the elution order was the same as that reported by Izumi [23]. For ^{13}C NMR spectral data see Table 1.

Determination of configuration of arabinose moiety in naturally occurring 6-O-α-*arabinofuranosyl-β-*D-*glucopyranosides* [11]. A standard soln of (+)2-BuOH [25] (300 μl) containing one drop of conc. H_2SO_4 was prepared. D- And L-arabinose were each warmed for 1 hr at 70–80° with this (+)2-BuOH soln (10 μl) and the reaction mixture then trimethylsilylated with 150 μl of reagent and analysed without further treatment. This established RR_t's and GC/MS characteristics of the individual diastereoisomeric (+)2-butyl arabinosides. The natural product, which had been purified as its acetate derivative, was deacetylated and the residue (6 mg) taken up in dil. HCl (*ca* 100 μl, pH 1.2). The aq. soln was treated at 100° for 30 min, cooled, extracted with Freon (3 × 200 μl), evaporated under N_2 and dried. The standard (+)2-BuOH soln (20 μl) was added to the residue which was heated at 70–80° for 2 hr, then trimethylsilylated with 200 μl of reagent. GC/MS analysis [conditions as in (f) including cochromatography] with the authentic glycosides prepared above, demonstrated that the arabinose in the natural product had the L-configuration.

Enzyme experiments. Emulsin (EC 3.2.1.21) (24 mg) and Rohapect C (a commercially available enzyme preparation from Röhm) (80 mg) were each made up in NaPi buffer (25 ml) at pH 5.0 and 4.3 respectively. A standard soln of substrate was prepared by dissolving the precursor material isolated from Muscat of Alexandria juice (4 l.) in H_2O (5 ml) and further diluting 1 ml of this to 10 ml. Incubation mixtures were made up by diluting the substrate soln (1 ml) and enzyme soln (1 ml) in NaPi buffer (5 ml) (pH 4.3 for Rohapect C and pH 5.0 for emulsin). These solns were then held at 37° for 16 hr. Controls, prepared as above but using enzyme inactivated by heating at 85° for 10 min, were also run. Liberated monoterpenes were extracted from the solns with Freon and analysed by GC and GC/MS. The extent of hydrolysis brought about by the enzymes was assessed by GC comparison of the products yielded by the substrate with the active enzymes and those obtained from acid hydrolysis.

Acknowledgements—We thank Dr. P. A. J. Gorin, National Research Council of Canada, for a reference sample of hepta-*O*-acetyl-6-*O*-α-L-arabinofuranosyl-β-D-glucose and Professor S. J. Angyal of the University of N.S.W. for a sample of methyl-β-D-arabinopyranoside as well as helpful discussion.

We are grateful to Dr. M. E. Tate, Waite Institute, University of Adelaide for assistance with the electrophoresis studies and Dr. M. E. Evans, The Australian Wine Research Institute for preparation and separation of the methyl arabinoside isomers.

The South Australian Department of Agriculture, Lindemans Wines and Pty. Ltd. and S. Smith and Sons Pty. Ltd. generously donated wines and grape juices for this study.

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