Phytochemistry, Vol. 31, No. 12, pp. 4307–4312, 1992
Printed in Great Britain.

0031-9422/92 $5.00+0.00

LIGNAN AND PHENYLPROPANOID GLYCEROL GLUCOSIDES IN WINE

VASSILIOS A. MARINOS, MAX E. TATE and PATRICK J. WILLIAMS*

Department of Plant Science, Waite Agricultural Research Institute, The University of Adelaide, Glen Osmond, SA 5064, Australia; *The Australian Wine Research Institute, P.O. Box 197, Glen Osmond, SA 5064, Australia

(*Received* 1 *April* 1992)

Key Word Index—*Vitis vinifera*; Vitaceae; Riesling wine; 2-[2-methoxy-4-(ω-hydroxypropyl)-phenoxy]-propan-1,3-diol; isolariciresinol; *seco*-isolariciresinol; β-D-glucopyranoside; fast atom bombardment-mass spectrometry.

Abstract—Three glycoconjugates, 1-*O*-(β-D-glucopyranosyl)-2-[2-methoxy-4-(ω-hydroxypropyl)-phenoxy]-propan-3-ol, 1,2,3,4-tetrahydro-7-hydroxy-1-(4′-β-glucopyranosyl-3′-methoxyphenyl)-6-methoxy-2,3-naphthalenedimethanol (isolariciresinol-β-4′-*O*-glucopyranoside) and, tentatively, 2,3-*bis*(4′-hydroxy-3′-methoxybenzyl)-butane-1,4-diol-β-D-glucoside (*seco*-isolariciresinol-β-D-glucoside) have been detected in Riesling wine by FAB-mass spectrometry/mass spectrometry and subsequently isolated and identified by spectral and chemical studies. The first of these compounds is a new shikimic acid metabolite of wine, and the latter two lignans represent a new category of wine phenols.

INTRODUCTION

Fast atom bombardment mass spectrometry (FAB-MS) and tandem mass spectrometry (MS/MS) experiments have been used to advantage in the study of wine glycosides, because of the ability of the combined techniques to provide structurally significant information from the daughter and parent ion relationships. These features have enabled the characterization of individual glycoconjugates in partially fractionated samples [1]. This paper describes the application of FAB-MS/MS to reveal the presence of two new classes of phenolic derivatives in wine.

RESULTS AND DISCUSSION

A series of polar glycosidic fractions was obtained from a Riesling wine by droplet counter current chromatography (DCCC, fr. 90–100), followed by flash chromatography on silica gel [2]. Monitoring these flash fractions by positive and negative FAB-MS indicated the presence of three glycoconjugates with apparent M_rs of 418, 522, 524. The M_r of each of these glycoconjugates was deduced from the presence of protonated and pseudo-molecular ions in the positive ion mode at m/z 419 $[M+H]^+$, 441 $[M+Na]^+$, 523 $[M+H]^+$, 545 $[M+Na]^+$ and 525 $[M+H]^+$ and deprotonated molecular ions in the negative ion mode at m/z 417 $[M-H]^-$, 521 $[M-H]^-$ and 523 $[M-H]^-$, respectively. FAB-MS/MS experiments on these protonated and deprotonated molecular ions provided the following structural information.

For the glycoside with apparent M_r 418 (glycoside **1**) MS/MS data suggested a monohexoside, which showed positive and negative mode fragment ions for the hexose ring at m/z 163 $[(\text{hexose}+H)-H_2O]^+$, 145 $[(\text{hexose}+H)-2H_2O]^+$, 127 $[(\text{hexose}+H)-3H_2O]^+$, and 161 $[(\text{hexose}-H)-H_2O]^-$, 119 $[(\text{hexose}-H-H_2O)-CH_2=C=O]^-$, 101, 89, respectively [3, 4]. The fragmentation pattern of the glycoside in the positive ion mode showed subsequent losses from the protonated molecular ion of 162 (loss of anhydrohexose) and of 74 leading to a protonated ion at m/z 183. In the negative mode losses were observed from the deprotonated molecular ion of 182 and 74 leading to an ion of a deprotonated anhydrohexose at m/z 161. These patterns are consistent with a molecule comprising three individual parts, namely, the hexose moiety, a segment of 182 m.u. and a third segment of 92 m.u.; the latter loses water to yield the observed fragment of m/z 74. Furthermore, either the hexose moiety or the segment of 182 m.u. were lost from the protonated and the deprotonated molecular ions, respectively, leaving alternatively the segment of 182 m.u. or the hexose moiety as the ultimate daughter ion. In either case the penultimate daughter ion showed a loss of 74 m.u. These data suggested a linear pattern of substitution with the segment of 92 m.u. being a disubstituted species. A candidate for this central disubstituted species was glycerol and a possible structure for **1** was, therefore, a glycosylated glycerol with a further substituent of apparent M_r 182. The fact that the protonated ion at m/z 183 in the positive mode daughter ion spectrum further generated a strong fragment ion at m/z 137, which is characteristic of a hydroxy-methoxy-substituted benzylium ion, suggested that the substituent could be dihydroconiferyl alcohol.

In the case of compounds **2** and **3** with apparent M_rs of 522 and 524, respectively, the positive and negative mode daughter ion mass experiments indicated that they were also monohexosides. Thus, glycoside **2** showed daughter ions in the positive ion mode at m/z 361 $[(M+H)-\text{anhydrohexose}$, i.e. $\text{aglycone}+H]^+$, 163 $[(\text{hexose}+H)-H_2O]^+$, 145 $[(\text{hexose}+H)-2H_2O]^+$ and in the negative ion mode daughter ions m/z 359 $[(M-H)-\text{anhydrohexose}]^-$ and 161 $[(\text{hexose}-H)-H_2O]^-$. For glycoside **3** a loss of an anhydrohexose unit from the protonated molecular ion in the positive mode, and a loss of a hexose

unit from the deprotonated molecular ion in the negative mode, were also observed.

Preparative separation by HPLC of the Riesling fraction which contained glycosides **1** and **2** (flash fractions 10–12), allowed isolation of these two glycosides. The UV absorbance of glycoside **1** showed λ_{max} at 278 nm, consistent with the presence of a substituted benzene ring [5]. The ^{1}H NMR spectrum (Table 1) showed the presence of three aromatic protons (δ6.86–7.07), six protons characteristic of a phenylpropanol side chain (two broad triplets at δ2.64, and 3.60 and a multiplet at δ1.84), signals for a glycerol moiety (δ3.27–4.55), a methoxyl singlet at δ3.86 and a signal for an anomeric proton of β-configuration (δ4.47). The above spectral data are essentially identical with those reported for 1-*O*-(β-D-glucopyranosyl)-2-[2-methoxy-4-(ω-hydroxypropyl)-phenoxy]-propan-3-ol [6].

Acetylation of **1** with acetic anhydride/pyridine produced the hexaacetate **1a** {$[M]^+$ *m/z* 670 (EIMS), $[M+H]^+$ *m/z* 671 (FAB-MS), $[M-H]^-$ *m/z* 669 (negative ion NH_4 CIMS)}, the positive ion spectra (EIMS, FAB-MS) of which contained a sequence of fragment ions at *m/z* 331, 271, 169, 109 characteristic of a monosubstituted tetra-*O*-acetylated hexopyranoside [7]. The observation in the EIMS and FAB-MS/MS of **1a** of ions at *m/z* 447 and 448 was consistent with fragmentation resulting from loss of the dihydroconiferyl alcohol monoacetate moiety from the $[M]^+$ and from the protonated molecular ion, respectively, as was suggested by the MS/MS study of **1**.

The ^{1}H NMR signals of the hexaacetate **1a** are given in Table 1; absence of a low field signal near δ2.3 for a phenolic acetate indicated that the dihydroconiferyl alcohol moiety was linked to the glycerol via the phenolic hydroxyl. The absence of a significant downfield shift after acetylation for the C-2 proton of the glycerol skeleton, indicated that the hydroxyl group on this centre was substituted. Furthermore, its chemical shift (δ4.45) suggested a CH-O-Ar system [5, 6], and therefore it was concluded that the dihydroconiferyl alcohol was attached to glycerol at position 2 as an aromatic ether. The signals observed for the sugar part of the molecule were in agreement with those previously published for tetra-acetylated glucopyranoses [8] and clearly established the sugar part of **1a** as tetra-acetylated glucopyranose with the anomeric proton having a β-configuration. The above spectral data for **1a** are in good agreement with those published previously by Miki *et al.* [6] for the per-*O*-acetylated 1-*O*-(β-D-glucopyranosyl)-2-[2-methoxy-4-(ω-hydroxypropyl)-phenoxy]-propan-3-ol. From ^{1}H–^{1}H-COSY of **1a** the coupling relationships of the individual protons were observed and these, together with data from decoupling experiments of the signals at δ4.08 (Table 1), were found to accommodate the proposed structure.

Enzymic hydrolysis of **1** with a β-D-glucosidase liberated an aglycone with an apparent M_r at *m/z* 256. This was deduced from the observation of the $[M]^+$ in the probe EI spectrum and from the observation of the protonated $[M+H]^+$, pseudomolecular $[M+Na]^+$ and the deprotonated $[M-H]^-$ molecular ions in the positive and negative FAB mass spectra, respectively. The MS/MS examination of the molecular ion (probe EI mode) produced a spectrum in agreement with that published for 2-[2-methoxy-4-(ω-hydroxypropyl)-phenoxy]-propan-1,3-diol [6]. Furthermore, the FAB-MS/MS of the $[M+H]^+$ generated fragment ions similar to those observed in the MS/MS of **1** for the aglycone portion. Finally, acid hydrolysis of **1**, followed by GC and GC-MS examination of the acid hydrolysis products as their TMSi derivatives, independently confirmed the sugar part as glucose. Thus, it was concluded that **1** is the 1-*O*-(β-D-glucopyranosyl)-2-[2-methoxy-4-(ω-hydroxypropyl)-phenoxy]-propan-3-ol.

Phenylpropanoid-glycerol glycosides have been reported to be components of lily bulbs [9], and 1-*O*-(β-D-glucopyranosyl)-2-[2-methoxy-4-(ω-hydroxypropyl)-phenoxy]-propan-3-ol has been isolated from the inner bark of *Larix leptolepis* [6]. Because phenylpropanoids are important intermediates in the biosynthesis of lignans and lignins [10], the role of phenylpropanoid-glycerol glycosides as potential membrane lipid structural entities is a matter of considerable interest.

Glycoside **2** showed absorption in its UV spectrum at λ_{max} 280, consistent with the presence of a substituted benzene ring [5]. The ^{1}H NMR spectrum (Table 1), showed signals for five aromatic protons (δ6.43–6.92) with only two of them showing a coupling relationship (J = 8.0 Hz, *ortho*-coupling), indicating the probable presence of two aromatic systems with one aromatic ring being tri- and the other one tetrasubstituted. Additionally, signals for two benzylic protons (δ2.83), one homobenzylic (δ2.05), one *bis*-homobenzylic (δ1.81), one anomeric proton with β-configuration (δ4.52, J = 7.5 Hz) and two methoxyl groups were observed. The data support the assignment of **2** as a glycoside of (+) and/or (−) isolariciresinol, and were in good agreement with data published previously for glycosylated derivatives of both these compounds [11, 12]. At this stage the data did not permit an assignment of the position of glycosylation, because the four hydroxy methylene protons of the aliphatic side chains of isolariciresinol could not readily be distinguished from the hydroxy methylene group of the carbohydrate portion of the molecule (δ3.32–3.75, 9H).

Acetylation of **2** with acetic anhydride/pyridine produced a heptaacetate **2a** ($[M+NH_4]^+$ *m/z* 834, $[M-H]^-$ *m/z* 815 positive and negative ion mode NH_4 CIMS); the ^{1}H NMR data are given in Table 1. The observation of only one phenolic acetate (δ2.29) suggested that **2a** was glycosylated via one of its phenolic hydroxyls. The upfield shift for one of the methoxyls (δ3.79 from 3.83), and one of the aromatic protons (δ6.61 from 6.92) as a consequence of acetylation, implied that they both were situated next to the acetylated phenolic hydroxyl. The absence of any detectable coupling between that aromatic proton which had shifted and any other proton in the ^{1}H–^{1}H NMR COSY experiment indicated that the aromatic proton was proximal to the fused ring. This suggested that the phenolic acetate was located at position C-7 of the tetrasubstituted ring of **2a**, and that the isolariciresinol was therefore glycosylated at position C-4′ of the other aromatic ring. Other signals observed for the aglycone part of **2a** were similar to those previously published for isolariciresinol acetate [12]. Furthermore, the exact chemical shifts and coupling relationships as deduced from the COSY ^{1}H–^{1}H NMR experiment verified **2a** as a glycosidic derivative of isolariciresinol. The signals observed for the saccharide portion of **2a** were second order and the coupling constant for the protons of the sugar portion was not determined.

Glycoconjugate **2** was resistant to enzymic treatment with β-D-glucosidase from sweet almond, but on enzymic hydrolysis with a fungal enzyme preparation (Novoferm

Table 1. ^{1}H NMR data of compounds **1**, **1a**, **2** and **2a** (300 MHz, δ)

H	**1** (D_2O)	**1a** ($CDCl_3$)	**1a** ^{1}H–^{1}H COSY	H	**2** (D_2O)	**2a** ($CDCl_3$)	**2a** ^{1}H–^{1}H COSY
1a	3.27, *br t*, $J=8.9$ Hz	4.08, *br t*, $J=6.5$ Hz*	H-1b, H-2	1	3.32–3.75, *m, obsc.*	3.91, *d*, $J=10.7$ Hz	H-2
1b	3.27, *br t*, $J=8.9$ Hz	3.80, *dd*, $\Sigma J=10.6$ Hz	H-1a, H-2	2	1.81, *br t*, $J=9.1$, 11.0 Hz	2.05, *m* (overlap with acetates)	H-1/H-3
2	4.55, *m*	4.45, *m*	H-1a/H-1b/H-3a/H-3b	3	2.05, *m*	2.20, *m* (overlap with acetates)	H-4/H-2/H-9a/H-9b
3a	3.39, *m*	4.08, *br t*, $J=6.5$ Hz*	H-3b/H-2	4	2.83, *m*	2.85, *m*	H-3
3b	3.39, *m*	4.31, *br t*, $J=5.7$ Hz	H-3a/H-2	5	6.43, *br s*	6.47, *br s*	
3′	6.98, *br s*	6.71, *br s*		8	6.92, *br s*	6.61, *s*	
5′	7.07, *d*, $J=6.8$ Hz	6.67, *dd*, $J=7.6$, 1.5 Hz	H-6′/H-3′	9a	3.32–3.75, *m*	4.25, *dd*, $J=4.1$, 11.1 Hz	H-9b/H-3
6′	6.86, *d*, $J=7.0$ Hz	6.90, *d*, $J=7.6$ Hz	H-5′	9b	3.32–3.75, *m*	4.10, *m*	H-9a/H-3
7′	2.64, *br t*, $J=8.0$ Hz	2.63, *br t*, $J=7.6$ Hz	H-8′	9′a	3.32–3.75, *m*	4.25, *dd*, $J=4.1$, 11.0 Hz	H-9′b/H-3
8′	1.84, *m*	1.93, *m*	H-7′H-9′a/H-9′b	9′b	3.32–3.75, *m*	4.10, *m*	H-9′a/H-3
9′a	3.60, *br t*, $J=6.2$ Hz	4.08, *br t*, $J=6.5$ Hz*	H-9′a/H-8′	2′	6.79, *br s*	6.70, *d*, $J=1.7$ Hz	H-6′
9′b	3.60, *br t*, $J=6.2$ Hz	4.31, *br t*, $J=5.7$ Hz	H-9′a/H-8′	5′	6.90, *d*, $J=8.1$ Hz	6.96, d, $J=8.1$ Hz	H-6′
1″	4.47, *d*, $J=6.6$ Hz	4.65, *d*, $J=7.9$ Hz	H-2″	6′	6.74, *d*, $J=8.0$ Hz	6.65, *dd*, $J=8.1$, 1.7 Hz	H-5′/H-2′
2″	3.4–4.1, *m*	5.00, *dd*, $J=8.1$, 9.5 Hz	H-1″/H-3″	1″	4.52, *d*, $J=7.5$ Hz	4.53, *m*	H-2″
3″	3.4–4.1, *m*	5.22, *br t*, $J=9.5$ Hz	H-2″/H-4″	2″	3.32–3.75, *m*	5.11–5.26, *m*	H-1″/H-3″
4″	3.4–4.1, *m*	5.07, *br t*, $J=9.6$ Hz	H-3″/H-5″	3″	3.32–3.75, *m*	5.11–5.26, *m*	H-2″/H-4″
5″	3.4–4.1, *m*	3.65, *m*	H-4″/H-6″a/H-6″b	4″	3.32–3.75, *m*	5.11–5.26, *m*	H-3″/H-5″
6″a	3.4–4.1, *m*	4.26, *obsc.*	H-5′/H-6″b	5″	3.02, *m*	3.45, *m*	H-4″
6″b	3.4–4.1, *m*	4.07, *obsc.*	H-5″/H-6″a	6″a	3.32–3.75, *m*	3.82, *dd*, $J=10.0$, 1.9 Hz	H-6″b
MeO-	3.86, *s*	3.83, *s*	—		3.32–3.75, *m*	4.10, *m*	H-6″a
Acetates	—	2.01, 2.02, 2.06 (2), 2.17 (2)	—	MeO-	3.83, *s*	3.79, *s*	—
				MeO-	3.78, *s*	3.78, *s*	—
				Acetates		2.00, 2.02, 2.05, 2.08, 2.17 (2), 2.29	

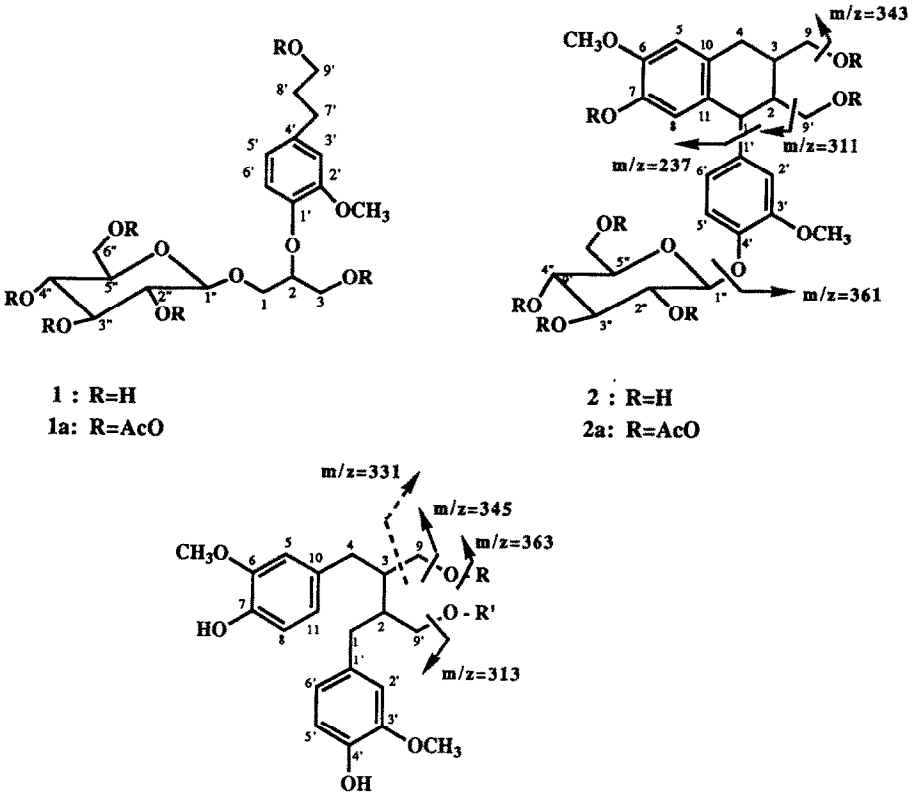
*Irradiation of signals at δ4.08 showed them to be coupled with signals at δ1.9 (8′), δ3.7 (5′), δ3.8 (1b), and δ4.3 (3b, 9′b).

12) liberated an aglycone with an apparent $[M]^+$ at m/z 360 (probe EIMS). Trimethylsilylation of the liberated aglycone and GC-MS examination of the derivative showed it as a single peak with a mass spectrum in close agreement with that previously published for the *per*-trimethylsilylated derivative of isolariciresinol [13]. On acid hydrolysis, **2** liberated a sugar which after trimethylsilylation was examined under GC and GC-MS conditions and found to be indistinguishable from glucose. Thus, glycoside **2** was characterized as the 1,2,3,4-tetrahydro-7-hydroxy-1-(4′-β-glucopyranosyl-3′-methoxyphenyl)-6-methoxy-2,3-naphthalenedimethanol (i.e. isolariciresinol-β-4′-*O*-glucopyranoside).

From a study of the fragmentation pathways of **2** under FAB-MS/MS conditions it was possible to assign the position of glycosylation in the molecule from that data also. Thus, parent ion experiments on the fragments observed in the positive mode daughter ion spectrum of **2** showed that only ions at m/z 361 and m/z 237 were directly associated with the $[M+H]^+$, suggesting formation of these ions after elimination of anhydroglucose or methoxyphenylglucose, respectively, from the $[M+H]^+$ (Fig. 1). This was consistent with isolariciresinol being glycosylated on the C-4′ phenolic hydroxyl. Further support for the assignment was provided by the negative mode daughter ion spectrum in which elimination of anhydroglucose but not of glucose from the $[M-H]^-$ of **2** was observed. This latter fragmentation was attributed to the fact that the negative charge was preferably stabilized on the phenolic hydroxyl rather than the aromatic ring, leading to cleavage of the glycoside between glucose and the phenolic oxygen only and to elimination of anhydroglucose.

Isolariciresinol glycosides have previously been isolated from the needles of *Pinus sylvestris* [12], *Pinus massoniana* [14], *Picea abies* [11] and *Populus nigra* [15]. In all cases reported, the glycosylation has involved the hydroxyls of the aliphatic side chains rather than the phenolic hydroxyls. This appears to be the first time that isolariciresinol has been identified as a phenolic glycoside.

The crude glycosidic flash fraction (fr. 6–7) containing the glycoside **3** was enzymically hydrolysed with β-D-glucosidase from sweet almonds. The liberated aglycones when examined by probe EIMS indicated the presence of a constituent with apparent M_r 362. Probe EI-MS/MS examination of that ion produced a daughter ion spectrum with the same fragments as those seen in the spectrum of a reference sample of 2,3-*bis*(4′-hydroxy-3′-methoxybenzyl)butane-1,4-diol (*seco*-isolariciresinol) when analysed under the same conditions. Trimethylsilylation of the aglycones and GC-MS examination revealed the existence of a peak which gave an identical EI spectrum to, and co-chromatographed with, the TMSi derivative of reference *seco*-isolariciresinol. Thus, it was concluded that the glycoconjugate **3** was a β-hexoside of



1 : R=H
1a: R=AcO

2 : R=H
2a: R=AcO

3: R=H or glucose
R'=glucose or H

Fig. 1. Structures of compounds **1**, **1a**, **2**, **2a** and **3** and some of their mass spectral fragmentations.

seco-isolariciresinol. HPLC separation of this fraction yielded pure glycoside **3**. The positive ion MS/MS experiment on the $[M+H]^+$ of **3** under FAB conditions showed, in addition to ions corresponding to fragmentation of the sugar ring, other ions at *m/z* 363, 345, 331 and 313, which appeared to be associated with the aglycone part of the molecule. It was unclear, however, whether these ions were derived from the protonated aglycone or the $[M+H]^+$. Parent ion experiments on these four ions showed that only those at *m/z* 363 and *m/z* 331 were directly associated with the $[M+H]^+$, arising after elimination of 162 (anhydrohexose) and 194 (methylhexoside), respectively (Fig. 1). This implied glycosylation of the aglycone via one of the aliphatic side chain hydroxyls rather than via one of the phenolic hydroxyls. This deduction was supported by the negative mode daughter ion spectrum where elimination of both anhydrohexose and hexose were observed, suggesting that fragmentation occurred on either side of the glycosidic oxygen. Such a fragmentation pathway was not observed in the negative ion spectrum of phenolic glycoside **2**. For the ions at *m/z* 345, 313 in the positive mode, the parent ion experiments suggested that they were formed after elimination of H_2O from ions at *m/z* 363 and 331, respectively.

On acid hydrolysis, **3** liberated a sugar which after trimethylsilylation was examined under GC conditions and found to be indistinguishable from glucose. Because **3** was present in the Riesling wine in only trace amounts, it was not possible to isolate sufficient material for a ^{1}H NMR study, and hence to confirm the position of glycosylation. Thus, compound **3** was tentatively assigned as the β-D-glucoside of *seco*-isolariciresinol with the sugar moiety being conjugated via one of the aliphatic rather than the phenolic hydroxyls.

seco-Isolariciresinol glycosides have previously been isolated from the needles of *Pinus sylvestris* [12] and from *Berchemia racemosa* [16]. In both cases, glycosylation has involved the hydroxyls of the aliphatic chains, as appears to be the case here. Although *seco*-isolariciresinol has never been detected in mammals, its glycoside has been implicated as a dietary precursor of *trans*-2,3-*bis*(3-hydroxybenzyl)-γ-butyrolactone (enterolactone) and 2,3-*bis*(3-hydroxybenzyl)butane-1,4-diol(enterodiol) [17] in urine. The latter are formed in the intestinal tract by bacteria [17] and their possible biological activities have been discussed [17, 18]. The presence of this mammalian and human lignan precursor in wine is of interest.

The spectrometric (^{1}H NMR, UV) verification of the initial assignments, which were based on an interpretation of FAB-MS/MS data for the molecules with M_r 418 and 522, demonstrates the utility of FAB-MS/MS for the detection and partial characterization of polar glycosidic compounds. This is the first time that the phenylpropanoid-glycerol glycoside **1** and the lignan glycosides **2** and **3** have been reported as wine constituents. It was observed that the enzymic hydrolysate of **1** had a strong caramel, buttery-like aroma raising the question of whether phenylpropanoid glycerols have an impact on wine flavour.

EXPERIMENTAL

Preparation of concentrates from 60 l of an Australian Riesling wine and DCCC resolution of them has been described previously [2].

Flash chromatography. Flash chromatography was carried out as described previously [19] employing the bottom phase of $CHCl_3$–MeOH–H_2O (7:13:8) as eluent. Using this system, early eluting material from DCCC (fr. 90–100, 65 mg) was sepd into 15 frs from which comb. frs 6–7 and comb. frs 10–12 were prepd for further purification by HPLC.

Thin-layer chromatography. TLC of underivatized glycosides was carried out as previously described [20]; this system was also employed to monitor the progress of enzymic hydrolyses.

HPLC resolution of glycosidic fractions. The analytical and semi-prep. work was done at 35° with reverse-phase C_{18} columns. Both analytical (25 cm, 4 mm i.d.) and semi-prep. (25 cm, 6.5 mm i.d.) columns were protected by a C_{18} precolumn. All solvents were HPLC grade, filtered through 0.45 mm membrane and ultrasonically degassed before use. Samples were membrane filtered (0.45 mm) before analysis. Elution was conducted at 1 ml min^{-1} (analytical) and 2 ml min^{-1} (semi-prep.) with a linear gradient of MeCN (A) and H_2O (B). Gradients, for the analytical work, were from 10% (A) to 20% (A) over 10 min and then held at that gradient for 5 min. For the semi-prep. work, gradients were from 10% (A) to 20% (A) over 15 min and from 20% (A) to 100% (A) over the next 5 min, and then held at that gradient for 8 min. Peak spectra were recorded at 210–400 nm with a diode array detector and detection was at 280 nm. HPLC sepn of the flash frs 6–7 (3 mg) gave a fr. containing **3** which eluted at a H_2O–MeCN gradient of 65.3:34.7. Flash frs 10–12 (19.7 mg) were sepd by chromatography on HPLC into 6 new frs with fr. 1 (eluted at a H_2O–MeCN gradient of 83.2:16.8) containing glucoside **1** (0.4 mg), and fr. 2 (eluted at a H_2O–MeCN gradient of 81.4:18.6) containing glucoside **2** (0.5 mg).

Gas chromatography. The chromatograph was equipped with either a 30 m J&W DB 5 fused silica column, 0.25 mm i.d. and 0.25 μm film thickness or with a SGE 25 m BP1 fused silica column, 0.33 mm i.d. and 0.5 μm film thickness, using He carrier gas at a linear velocity of 40 cm sec^{-1}. Injections were made using a split/splitless mode (1:10) at 280° (for per-*O*-acetyl- and TMSi derivatives). For analyses of peracetylated glycosides, the DB 5 column was held isothermal (5 min) at 100° followed by a prog. from 100° to 320° at 5° min^{-1}. For analysis of TMSi derivatives of sugars on the BP1 column, the prog. was 1 min isothermal at 120° followed by 120° to 180° at 2° min^{-1}, 1 min isothermally at 180° and finally from 180° to 300° at 7° min^{-1}. For analysis of TMSi derivatives of aglycones, the temp. prog. was 1 min isothermal at 120° followed by heating from 120° to 320° at 5° min^{-1}. The temp. of the detector (FID) was 280°.

Mass spectrometry. All analyses were made with a triple stage quadrupole MS, and unless otherwise stated at an electron beam energy of 70 eV.

Probe-EI mass spectrometry. In all cases the probe temp. was prog. from 100° to 350° at 100° min^{-1}.

Probe-CI mass spectrometry. The probe temp. was prog. from 100° to 350° at 100° min^{-1}, the source temp. was 160° and NH_3 was used as reagent gas.

Fast atom bombardment-mass spectrometry. Analyses were performed using Xe as bombardment gas. The established voltage was 7–10 keV, the ion current <0.5 mA. Glycerol or acidified glycerol (5% HOAc) were used as liquid matrices in the positive ion mode and glycerol in the negative ion mode. Typical sample concn was 1 mg ml^{-1}. During MS/MS expts the collision offset (COFF) varied between −15 and −30 eV (optimum conditions), the collision cell pressure was *ca* 19 Pa and the source temp. was 70°.

1-O-(β-D-*Glucopyranosyl*)-2-[2-*methoxy*-4-(ω-*hydroxypropyl*)-*phenoxyl*]-*propan*-3-*ol* (**1**). Yield 0.4 mg. TLC R_f 0.43. UV λ_{max} 280 nm. FAB-MS (positive ion mode): $[M+H]^+$ *m/z* 419, $[M+Na]^+$ *m/z* 441, (negative ion mode): $[M-H]^-$ *m/z* 417. FAB-MS/MS (positive ion mode): *m/z* 419 $[M+H]^+$, 257 $[(M+H)$

−anhydroglucose, i.e. aglycone+H]$^+$, 239 [(M+H) −anhydroglucose − H_2O]$^+$, 221 [(M+H)−anhydroglucose −2H_2O]$^+$, 183 [(aglycone+H)−anhydroglycerol]$^+$, 165 [(aglycone+H)−anhydroglycerol − H_2O]$^+$, 163 [(glucose +H)−H_2O]$^+$, 145 [(glucose+H)−2H_2O]$^+$, 137 [C_8H_9O, i.e. hydroxy−methoxy−benzylium]$^+$, 127 [(glucose+H) −3H_2O]$^+$; (negative ion mode): *m/z* 417 [M−H]$^-$, 235 [(M −H)−$C_{10}H_{14}O_3$]$^-$, 181 [(aglycone−H)−anydroglycerol]$^-$, 161 [(glucose−H)−H_2O]$^-$ (note, assignments are based on likely fragment ion compositions). Acetate **1a**. GC-EIMS: *m/z* (rel. int. %) 670 (3), 448 (89), 331 (3), 323 (4), 223 (5), 211 (2), 203 (5), 175 (9), 169 (100), 164 (13), 159 (23), 145 (19), 127 (17), 117 (14), 115 (14), 109 (54), 103 (14), 81 (13), 57 (7). FAB-MS: [M+H]$^+$ 671. FAB-MS/MS: *m/z* 671 [M+H]$^+$, 448 [(M+H) −$C_{12}H_{19}O_9$]$^+$, 331 [$C_{14}H_{19}O_9$]$^+$, 271 [$C_{12}H_{15}O_7$]$^+$, 211 [$C_{10}H_{11}O_5$]$^+$, 169 ($C_8H_9O_4$]$^+$, 109 [$C_6H_5O_2$]$^+$ (note, assignments are based on likely fragment ion compositions). ^{1}H NMR of compounds see Table 1.

Isolariciresinol-β-4′-O-glucopyranoside (**2**). Yield 0.5 mg (amorphous solid). TLC R_f 0.51. UV λ_{max} 280 nm. FAB-MS (positive ion mode) *m/z*: 523 [M+H]$^+$, 545 [M+Na]$^+$; (negative ion mode): 521 [M−H]$^-$. FAB-MS/MS (positive ion mode): *m/z* 523 [M+H]$^+$, 505 [(M+H)−H_2O]$^+$, 361 [(M+H)−anhydroglucose]$^+$, 343 [(aglycone+H)−H_2O]$^+$, 311 [(aglycone +H)−H_2O−MeOH]$^+$, 237 [(M+H)−anhydroglucose −$C_7H_8O_2$]$^+$, 219 [(M+H)−anhydroglucose−$C_7H_8O_2$ −H_2O]$^+$, 163 [(glucose+H)−H_2O]$^+$, 145 [(glucose+H) −2H_2O]$^+$, 137 [$C_8H_9O_2$]$^+$; (negative ion mode): 521 [M −H]$^-$, 359 [aglycone−H]$^-$, 161 [(glucose−H)−H_2O]$^-$ (note, assignments are based on likely fragment ion compositions). Compound **2a**: NH_4-CIMS: *m/z* [M+NH_4]$^+$ 834, [M−H]$^-$ 815. ^{1}H NMR data see Table 1.

seco-*Isolariciresinol-β-*D*-glucoside* (**3**). UV λ_{max} 210 (sh), 280 nm. FAB-MS (positive ion mode): *m/z* 525 [M+H]$^+$, (negative ion mode): 523 [M−H]$^-$. FAB-MS/MS (positive ion mode): *m/z* 525 [M+H]$^+$, 363 [(M+H)−anhydroglucose]$^+$, 345 [(aglycone+H)−H_2O]$^+$, 331 [(M+H)−$C_7H_{14}O_6$ (i.e. methylhexose)]$^+$, 313 [(aglycone+H)−MeOH−H_2O]$^+$, 287, 222, 178, 146, (aglycone fragments); (negative ion mode): 523 [M −H]$^-$, 343 [aglycone−H]$^-$ (note, assignments are based on likely fragment ion compositions).

Hydrolyses of glycosides. (*a*) *Enzymic hydrolyses.* Enzymic hydrolyses of glycoconjugates **1–3** were carried out as described previously [2] but using the fungal enzyme preparation Novoferm 12 (Novo Nordisk) and sweet almond β-D-glucosidase (Serva, EC 3.2.1.21). The liberated aglycones were examined by probe EI-MS/MS and FAB-MS/MS (aglycone of glucoside **1**), by EI-MS/MS (*seco*-isolariciresinol) and by GC-MS as their TMSi derivatives (isolariciresinol and *seco*-isolariciresinol) and showed the following previously unpublished spectral data. Aglycone of **1**: EI-MS (probe): *m/z* [M]$^+$ 256; FAB-MS: *m/z* [M +H]$^+$ 257, [M+Na]$^+$ 279, [M−H]$^-$ 255. FAB-MS/MS: *m/z* 257 [aglycone+H]$^+$, 183 [(aglycone+H)−anhydroglycerol]$^+$, 165 [(aglycone+H)−anhydroglycerol−H_2O]$^+$, 137 [C_8H_9O, i.e. hydroxy-methoxy-benzylium]$^+$, 133 [C_8H_9O−MeOH]$^+$ (note, assignments are based on likely fragment ion compositions). *seco*-Isolariciresinol. EIMS (as TMSi derivatives) *m/z*: (rel. int. %) 650 (1), 560 (1), 470 (2), 439 (2), 424 (1), 274 (2), 261 (35), 248 (13), 209 (100), 179 (22), 147 (9), 103 (8), 73 (9).

(*b*) *Acid hydrolyses.* Acidic solns (0.035% perchloric acid aq. soln, pH 2.5) of the glycosides were heated at 100° for 15 min. The solns were neutralized (equivalent molarity KOH), dried (under N_2), desiccated overnight and, after prepn of the TMSi derivatives, the samples were examined by GC or GC-MS as described above.

Acknowledgements—We thank Dr R.C. Cambie of the University of Auckland, New Zealand, for suppling a sample of *seco*-isolariciresinol. The Grape and Wine Research and Development Corporation are thanked for supporting this research.

REFERENCES

1. Marinos, V. A., Tate, M. E. and Williams, P. J. (1992) *Phytochemistry* (in press).
2. Winterhalter, P., Sefton, M. A. and Williams, P. J. (1990) *J. Agric. Food Chem.* **38**, 1041.
3. Pare, J. R. J., Jankowski, K. and ApSimon, J. W. (1988) *Adv. Heterocy. Chem.* **42**, 335.
4. Domon, B. and Costello, C. (1988) *Biochemistry* **27**, 1534.
5. Williams, D. and Fleming, I. (1987) in *Spectroscopic Methods in Organic Chemistry*, p. 24. McGraw-Hill, Maidenhead, England.
6. Miki, K. and Sasaya, T. (1979) *Mokuzai Gakkaishi* **25**, 361.
7. Radford, T. and Dejongh, D. C. (1972) in *Biochemical Applications of Mass Spectrometry* (Waller, G. R., ed.), pp. 313–350. Wiley-Interscience, N.Y.
8. Schwab, W. and Schreier, P. (1988) *Phytochemistry* **27**, 1813.
9. Shimomura, H., Sashida, Y., Mimaki, Y., Kudo, Y. and Maeda, K. (1988) *Chem. Pharm. Bull.* **36**, 4841.
10. Mann, J. (1980) in *Secondary Metabolism*, p. 161. Oxford Chemistry Series.
11. Lundgren, L. N., Popoff, T. and Theander, O. (1981) *Phytochemistry* **20**, 1967.
12. Popoff, T. and Theander, O. (1977) *Acta Chem. Scand.* **B 31**, 329.
13. Kraus, R. and Spiteller, G. (1990) *Phytochemistry* **29**, 1653.
14. Lundgren, N. L., Shen, Z. and Theander, O. (1985) *Acta Chem. Scand.* **B 39**, 241.
15. Benecke, R., Nishibe, S., Kitagawa, S. and Hanschmann, H. (1989) *Pharmazie* **44**, 357.
16. Inoshiri, S., Sasaki, M., Kohda, H., Otsuka, H. and Yamasaki, K. (1987) *Phytochemistry* **26**, 2811.
17. Axelson, M., Sjövall, J., Gustafsson, B. E. and Setchell, K. D. R. (1982) *Nature* **298**, 659.
18. Adlercreutz, H. (1984) *Gastroenterology* **86**, 761.
19. Still, W. C., Kahn, M. and Mitra, A. (1978) *J. Org. Chem.* **43**, 2923.
20. Strauss, C. R., Gooley, R. P., Wilson, B. and Williams, P. J. (1987) *J. Agric. Food Chem.* **35**, 519.