Acid-Catalyzed Hydrolysis of Alcohols and Their β-D-Glucopyranosides†

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The hydrolysis, in model wine at pH 3, of the allylic, homoallylic, and propargylic glycosides, geranyl-β-D-glucopyranoside, [3′-(1″-cyclohexenyl)-1′-methyl-2′-propynyl]-β-D-glucopyranoside, (3′*RS*,9′*SR*)-(3′-hydroxy-5′-megastigmen-7-yn-9-yl)-β-D-glucopyranoside, (3′,5′,5′-trimethyl-3′-cyclohexenyl)-β-D-glucopyranoside, *E*-(7′-oxo-5′,8′-megastigmadien-3′-yl)-β-D-glucopyranoside (3-hydroxy-β-damascone-β-D-glucopyranoside), and their corresponding aglycons has been studied. In general, aglycons were more rapidly converted to transformation products than were the corresponding glucosides. Glycoconjugation of geraniol in grapes is a process that reduces the flavor impact of this compound in wine, not only because geraniol is an important flavor component of some wines but also because the rate of formation of other flavor compounds from geraniol during bottle-aging is reduced. However, when flavor compounds such as β-damascenone are formed in competition with flavorless byproducts, such as 3-hydroxy-β-damascone, by acid-catalyzed hydrolytic reactions of polyols, then glycoconjugation is a process that could enhance as well as suppress the formation of flavor, depending on the position of glycosylation. (3′*RS*,9′*SR*)-(3′-Hydroxy-5′-megastigmen-7′-yn-9′-yl)-β-D-glucopyranoside hydrolyzed more slowly but gave a higher proportion of β-damascenone in the products than did the aglycon at 50 °C. Reaction temperature also effected the relative proportion of the hydrolysis products. Accelerated studies do not parallel natural processes precisely but only approximate them.

Keywords: *β-Damascenone; geraniol; monoterpenes; norisoprenoids; hydrolysis; glycosides; precursors*

INTRODUCTION

In wines and other beverages, the development of flavor is of great importance to the consumer. Flavor is derived from a range of compounds, differing in chemical functionality. These compounds, which originate from primary or secondary metabolic pathways in grape berries or other fruits, can be formed by many routes such as the mevalonic acid, shikimate, and polyketide pathways as well as from carotenoid breakdown products (Ohloff et al., 1973; Sefton et al., 1989; Williams et al., 1992; Razungles et al., 1993). Many compounds apparently important to wine flavor such as β-damascenone (**1**) are thought to be derived from the hydrolytic breakdown of grape-derived conjugates (mainly glycoconjugates) of complex secondary metabolites (Francis et al., 1992, 1999,; Sefton et al., 1993, 1994; Winterhalter and Skouroumounis, 1996), yet little is known about the comparative hydrolytic behavior of such conjugates and their corresponding aglycons.

This paper reports the mild acid-catalyzed hydrolyses of some model and naturally occurring allylic, homoallylic, and propargylic alcohols and their β-D-glucopyranosides. The synthesis of these β-D-glucopyranosides has been described previously (Skouroumounis et al., 1995). Some of the hydrolytic data have also been reported in a preliminary form (Skouroumounis et al., 1992, 1993).

EXPERIMENTAL PROCEDURES

Preparation of Substrates. The alcohols (**2**−**6**) and the corresponding β-D-glucopyranosides (**2a**−**6a**) (Figure 1) were obtained or synthesized as described previously by Skouroumounis et al. (1995) except that 3-hydroxy-β-damascone (**6**) was synthesized by refluxing the acetylenic triol (**26**), (Massy-Westropp et al., 1992) in 0.1 N HCl for 1 h. The reaction mixture was cooled and extracted with dichloromethane. The aqueous layer was then salted out (sodium chloride), unreacted acetylenic triol was extracted with ethyl acetate and dried, and the above procedure was repeated until only trace amounts of acetylenic triol (**26**) were left. The dichloromethane extracts were combined and evaporated, and the oil was directly chromatographed under standard conditions (Still et al., 1978) using diethyl ether as solvent, giving 3-hydroxy-β-damascone (**6**) as a clear oil (70%) and β-damascenone (**1**) (16%). 1-(1′-Cyclohexenyl)-2-buten-1-one **(21)** was synthesized according to the method of Richter and Otto (1990). All chiral substrates used in the hydrolytic studies were racemates.

Preparation of Hydrolysates. Hydrolytic studies of substrates were carried out with 10% aqueous ethanolic solutions (model wines). The hydrolyses were conducted at pH 1.0, 1.1, or 3.0 in sealed ampules. Typical wine pH varies from 2.8 to 4.0. Model wine hydrolyses were conducted at pH 3.0. Before the ampules were sealed, the solutions were deoxygenated with a stream of oxygen-free dry nitrogen (Shriver, 1969) for 15 s. All hydrolyses were done in duplicate. A constant-temperature oven (Contherm Incubator) was used for experiments conducted at 50 °C over extended periods (>2 days). The temperature was monitored with a standard thermometer. Constant-temperature oil baths were used at 50, 80, or 100

† The authors dedicate the manuscript to the memory of the late Evangelos Cotsaris, a fellow scientist.

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10.1021/jf9904970 CCC: $19.00
Published on Web 05/13/2000

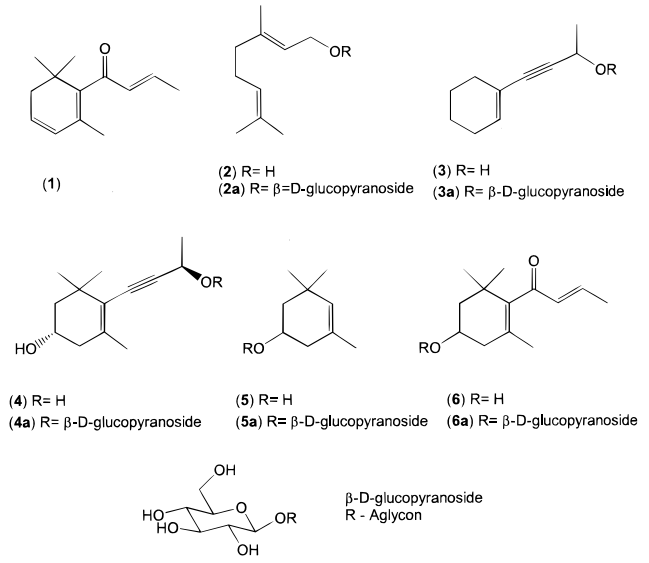


Figure 1. Aglycons and their β-D-glucosides. Relative stereochemistry only.

°C for hydrolyses carried out over shorter periods. The hydrolytic media were made up as follows: model wines at pH 1.0; (+)-tartaric acid was dissolved in 10% aqueous ethanol, and the pH was adjusted with sulfuric acid (1 M); pH 1.1, 0.1 N HCl solution; pH 3.0, 10% aqueous ethanol was saturated with potassium hydrogen tartrate, and the pH was adjusted with sulfuric acid (1 M); pH was determined with a pH meter, standardized with a reference solution (Weast, 1979−1980). For D_2O experiments, pH 1.0 or 3.0, tartaric acid was dissolved in D_2O and the water was evaporated under reduced pressure. This was repeated twice. Finally, the tartaric acid (d_4) was dissolved in D_2O and the pH adjusted with D_2SO_4 for pH 1.0 and with anhydrous potassium carbonate for pH 3.0. The aglycon (**6**) used for hydrolytic studies in D_2O was dissolved in deuterated ethanol (EtOD), and solvent was evaporated. This was repeated three times to ensure complete deuterium exchange. Finally, hydrolysis was carried out under model wine conditions.

The following amounts of aglycon or glycoside (milligrams per milliliter of hydrolytic medium) and internal standard (I.S.) were used: geraniol(**2**) or its β-D-glucopyranoside (**2a**) (each 1 mg/mL) with 2-phenylethanol (I.S., 1 mg/mL); enyne alcohol (**3**), the β-D-glucopyranoside (**3a**), 3,5,5-trimethyl-3-cyclohexen-1-ol (**5**), or the β-D-glucopyranoside (**5a**) (each 1 mg/mL, with *n*-butyl benzene [I.S., 1 mg/mL]); enynediol (**4**) (0.5 mg/mL), the β-D-glucopyranoside (**4a**) (2 mg/mL), or the β-D-glucopyranoside (**6a**) (1 mg/mL), each with octadecanol (I.S., 1 mg/mL). All internal standards were added as dichloromethane solutions after hydrolysis but before workup.

Analysis of Hydrolysates. Hydrolysates were worked up by cooling the ampules, adding the internal standard, and then extracting with dichloromethane (2 × 1 mL). The dichloromethane layer was washed with saturated sodium hydrogen carbonate (1 mL), dried ($MgSO_4$), filtered, and injected directly into the GC for analysis. For the hydrolyses of enynediol (**4**) the dichloromethane extract was concentrated by distillation through a column of Fenske's helices prior to analysis.

Analyses were performed with a Varian 3300 gas chromatograph coupled to either a Finnigan TSQ 70 mass spectrometer or a flame ionization detector. The column used was a 30 m J&W DB-1701 fused silica column (0.25 mm i.d. and 0.25 μm film thickness) with helium carrier gas at a linear velocity of 40 cm/s. Injections were made with the injector at 200 °C, and the splitter, with a split ratio of 1:10, was opened after 0.3 min. Flame ionization detection was used for compound quantification with a Hewlett-Packard autointegration system. For identification, hydrolysates were analyzed by GC-MS under the following conditions: The column was held for 1 min at 50 °C or at 100 °C for compounds **4**, **4a**, **6** and **6a**, then programmed at 4 °C/min to 250 °C, and held at that temperature for 20 min. Electron impact spectra were taken at 70 eV.

Table 1. Products of the Hydrolysis of Geraniol (2) at 50 and 80 °C, pH 3, in 10% Aqueous Ethanol

	50 °C yields (%)				80 °C yields (%)			
product[a]	1 h	4 h	8 h	24 h	1 h	4 h	8 h	24 h
2,2,6-trimethyl-2-vinyl-tetrahydropyran (**7**)[b]	−	−	−	+	0.1	0.3	0.9	3.7
myrcene (**8**)[b]	+	0.1	0.1	0.2	0.2	0.5	0.8	1.7
limonene (**9**)[b]	−	−	−	+	0.1	0.3	0.9	2.2
(*Z*)-ocimene (**10**)[b]	−	+	0.1	0.2	0.3	0.6	1.1	1.7
(*E*)-ocimene (**11**)[b]	−	0.1	0.1	0.2	0.3	1	1.8	2.7
terpinolene (**12**)[b]	−	−	−	+	0.1	0.3	1.1	1.8
linalool (**13**)	2.5	6.2	13	28	25	46	43	13
α-terpineol (**14**)	0.3	0.4	0.5	1.4	1.1	7.5	20	44
nerol (**15**)[b]	+	0.1	0.4	0.7	0.8	2	3.3	4.2
geraniol (**2**)	97	93	85	67	71	37	17	2.7
2,6-dimethyl-7-octene-2,6-diol (**16**)[b]	+	0.1	0.2	0.6	0.3	2.5	6.2	11
cis-1,8-terpin (**18**)[b]	−	−	−	−	−	−	0.5	4.3
trans-1,8-terpin (**17**)[b]	−	−	−	−	−	−	−	0.5
(*Z*)-3,7-dimethyl-2-octene-1,7-diol (**19**)[b]	+	+	0.1	0.1	+	0.2	0.7	2.9
(*E*)-3,7-dimethyl-2-octene-1,7-diol (**20**)[b]	−	−	0.7	1.6	0.6	2.3	2.5	3

[a] Assignments of all compounds have been established previously in this laboratory by Strauss (1983). [b] Concentration based on the assumption that components give the same GC-FID peakarea/mg as the internal standard. −, not detected; +, <0.1%. Coefficients of variation for all analyses were <12%.

Table 2. Products of Hydrolysis of Geraniol-β-D-glucopyranoside (2a) at 50 and 80 °C, pH 3, in 10% Aqueous Ethanol

	50 °C yields (%)				80 °C yields (%)			
product[a]	1 h	4 h	8 h	24 h	1 h	4 h	8 h	24 h
2,2,6-trimethyl-2-vinyl-tetrahydropyran (**7**)[b]	−	−	−	−	−	−	0.1	0.6
myrcene (**8**)[b]	−	−	−	−	−	−	0.1	−
limonene (**9**)[b]	−	−	−	−	−	−	0.1	0.3
(*Z*)-ocimene (**10**)[b]	−	−	−	−	−	−	0.1	0.2
(*E*)-ocimene (**11**)[b]	−	−	−	−	−	0.1	0.1	0.3
terpinolene (**12**)[b]	−	−	−	−	−	−	−	0.3
linalool (**13**)	0.1	0.2	0.5	0.5	1.5	2.9	4.6	3.8
α-terpineol (**14**)	0.4	0.4	0.5	0.5	1	0.8	1.8	5.3
nerol (**15**)[b]	−	−	−	−	−	0.1	0.3	0.4
geraniol (**2**)	0.15	0.15	0.1	−	0.2	−	−	−
2,6-dimethyl-7-octene-2,6-diol (**16**)[b]	−	0.4	0.3	0.2	−	−	0.6	2.2
cis-1,8-terpin (**18**)[b]	−	−	−	−	−	−	−	0.3
trans-1,8-terpin (**17**)[b]	−	−	−	−	−	−	−	−
(*Z*)-3,7-dimethyl-2-octene-1,7-diol (**19**)[b]		−	−	−	−	−	−	0.3
(*E*)-3,7-dimethyl-2-octene-1,7-diol (**20**)[b]	−	−	−	−	−	−	−	−

[a] Assignments of all compounds have been established previously in this laboratory by Strauss (1983). [b] Concentration based on the assumption that components give the same GC-FID peakarea/mg as the internal standard. −, not detected. Coefficients of variation for all analyses were <12%.

Identification of Hydrolysis Products. ^{1}H NMR spectra were recorded with a JEOL JNM-PMX (60 MHz) or a Bruker CXP300 (300 MHz) spectrometer. All compounds in Tables 1 and 2 (Figure 2) have been identified previously by Strauss (1983). 1-(1′-Cyclohexenyl)-2-buten-1-one **(21)** was compared with the synthetic sample: ^{1}H NMR (60 MHz/$CDCl_3$) δ 7.1−6.6 (m, 3H), 2.4−2.2 (m, 4H), 1.9 (d, J = 5 Hz, 3H), 1.8−1.6 (m, 4H); *m/z* 151 (8), 150 (46), 149 (8), 136 (9), 135 (100), 122 (6), 121 (11), 117 (11), 109 (9), 108 (6), 107 (17), 93 (6), 91 (7), 81 (19), 79 (24), 77 (6), 69 (45), 53 (5), 41 (38), 39 (14). Compounds **29** and **30** were identified by comparison with authentic materials (Sefton et al., 1989). Compounds **22**, **24a**, **25a**, **27a**−**d**, and **28** were tentatively assigned on the basis of interpretation of their mass spectral data. Compound **22**: *m/z*

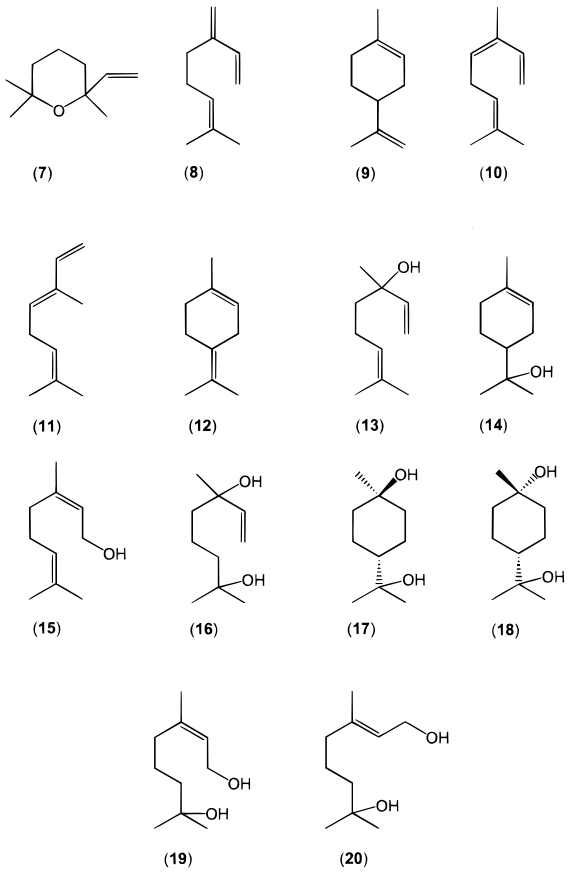


Figure 2. Products from hydrolysis of geraniol. Relative stereochemistry only.

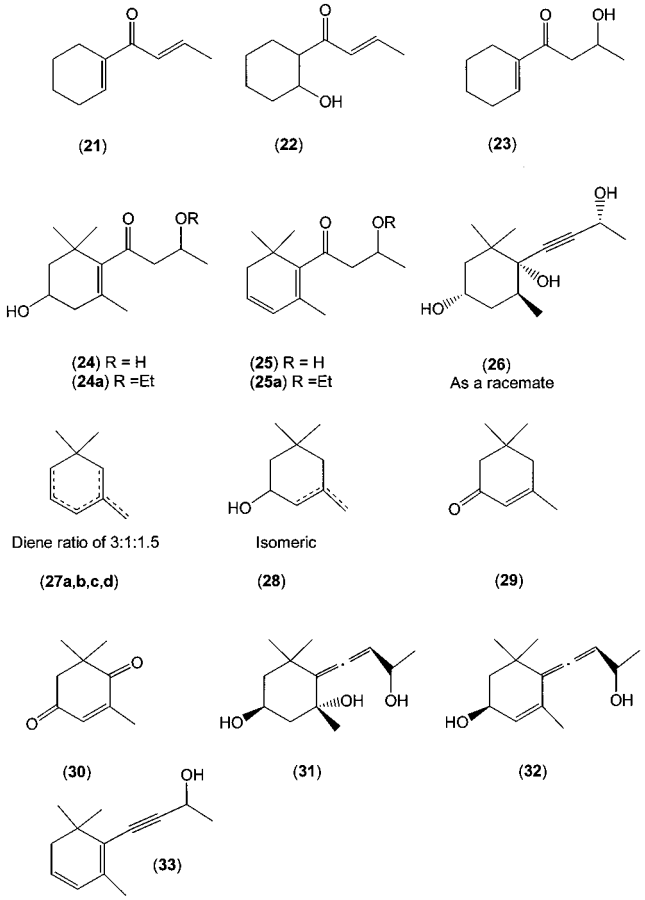


Figure 3. Compound numbers as referred to in text. Relative stereochemistry only.

168 (23), 153 (5), 150 (8), 126 (4), 125 (36), 124 (7), 99 (7), 98 (100), 83 (25), 81 (3), 79 (5), 77 (3), 71 (3), 70 (37), 69 (9), 67 (4), 56 (3), 55 (15), 53 (3). Ethyl ether **24a** (diastereoisomer 1) *m*/*z* 254 (8), 239 (8), 208 (13), 195 (11), 193 (6), 175 (5), 168 (13), 167 (100), 166 (8), 153 (6), 151 (18), 149 (10), 123 (8), 122 (9), 121 (34), 113 (7), 95 (7), 93 (9), 86 (8), 79 (7), 73 (35), 69 (14), 67 (7), 55 (14), 45 (51), 43 (20), 41 (14); (diastereoisomer 2) *m*/*z* 254 (7), 239 (8), 209, (9), 208 (20), 195 (10), 193 (13), 175 (8), 168 (13), 167 (100), 166 (9), 153 (8), 151 (20), 149(11), 140 (12), 139 (11), 138 (7), 126 (15), 125 (9), 123 (14), 122 (10), 121 (39), 113 (7), 107 (6), 95 (10), 93 (10), 91 (6), 81 (6), 79 (11), 77 (8), 73 (33), 69 (13), 67 (9), 55 (17), 45 (48), 43 (26), 41 (23). Ethyl ether **25a**: *m*/*z* 236 (7), 221 (7), 192 (3), 175 (5), 150 (5), 149 (51), 134 (3), 133 (36), 131 (3), 123 (11), 122 (100), 121 (59), 120 (8), 119 (9), 113 (6), 107 (25), 106 (4), 105 (19), 103 (5), 93 (4), 91 (14), 83 (7), 79 (10), 78 (3), 77 (9), 73 (44), 69 (5), 65 (4), 55 (3), 49 (4), 45 (57), 43 (8), 41 (9). Compound **27a**: *m*/*z* 122 (37), 107 (100), 91 (50), 79 (14), 49 (17). **27b**: *m*/*z* 122 (38), 107 (100), 105 (18), 91 (52), 84 (25), 79 (17), 77 (12), 51 (12). **27c**: *m*/*z* 122 (40), 107 (100), 93 (47), 91 (58), 86 (45), 79 (85), 77 (35), 66 (30), 49 (58). **27d**: *m*/*z* 122 (15), 107 (100), 105 (33), 92 (16), 91 (65), 86 (18), 84 (33), 79 (16), 77 (15), 68 (17), 49 (25). **28** *m*/*z* 140 (8), 125 (32), 109 (8), 107 (18), 97 (25), 82 (100), 69 (22), 55 (25). Compounds **24** and **25** have been tentatively identified previously (Sefton et al., 1989). 3-Hydroxy-β-damascone (**6**) at pH 1.0 for 8 h at 100 °C in D_2O/10% EtOD: ^{1}H NMR (300 MHz/$CDCl_3$) δ 6.75−6.65 (b, 1H), 6.14 (d, J = 15.8 Hz, 1H), 4.1 (m, 1H), 2.34 (ddd, J = 16.6, 5.9, 1 Hz, 1H), 2.0 (dd, J = 16.6, 9.9 Hz, 1H), 1.72 (m, 1H), 1.55 (s, 3H), 1.48−1.44 (m, 1H), 1.14 (s, 3H), 0.97 (s, 3H). The C10 methyl doublet at δ 1.72 was replaced by a complex multiplet resulting from partial deuterium incorporation at C10: *m*/*z* 211 (7), 210 (27), 209 (38), 208 (16), 195 (15), 194 (32), 193 (42), 177 (29), 176 (54), 175 (39), 150 (25), 149 (27), 122 (29), 121 (100), 107 (19), 105 (30), 93 (27), 91 (20), 79 (19), 72 (13), 71 (62), 70 (95), 69 (54), 55 (31). The above compounds are represented in Figure 3.

RESULTS AND DISCUSSION

Hydrolysis of Geraniol (2) and Its Glucopyranoside (2a). Hydrolysis of the simple allylic alcohol geraniol (**2**) and its glucopyranoside (**2a**) was carried out at a typical wine pH (3.0) at 50 and 80 °C. The results are shown in Tables 1 and 2.

The hydrolysis of geraniol led ultimately to 14 products observed by gas chromatography (Figure 2). These all increased in concentration with increasing temperature and reaction time except for linalool (**13**), which is an intermediate in the formation of α-terpeniol (**14**) and other products from geraniol (**2**) (Baxter et al., 1978). No ethyl ethers, including geraniol ethyl ether, were observed in the hydrolysates, even though the hydrolysis medium contained ethanol. The hydrolysis of many monoterpenes under a variety of acid conditions has previously been studied and reviewed by Baxter et al. (1978) and Strauss and Williams (1983). The pattern of products shown in Table 1 is consistent with results previously reported (Baxter et al., 1978; Ohta et al., 1991).

As a control for purity of starting material and for possible artifacts of analysis, geranyl-β-D-glucopyranoside (**2a**) was dissolved in 10% ethanol and the solution extracted with dichloromethane (2 mL). Analyses by GC-MS showed that 0.16% free geraniol (**2**) was present. Hydrolysis of the glucoside (**2a**) at 50 °C gave 3 products only (Table 2), linalool (**13**), a larger amount of α-terpineol (**14**), and 2,6-dimethyl-7-octene-2,6-diol (**16**), compared to the 13 compounds identified in the aglycon hydrolysis at this temperature. No additional geraniol (**2**) was observed other than that present in the control

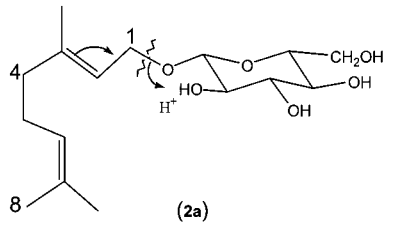


Figure 4. Proposed ionization of geraniol-β-D-glucopyranoside during acid hydrolysis at pH 3.0.

run. At 80 °C additional products were seen, along with linalool (**13**) and α-terpineol (**14**) as the major products. Again, no additional geraniol (**2**) other than that in the control was observed, suggesting that under these conditions primary allylic glucosides cleave at the ether linkage and not at the glycosidic bond (Figure 4).

Earlier studies reported by Williams et al. (1982) on the hydrolysis of geranyl-β-D-glucopyranoside (**2a**), under more vigorous conditions, suggested that a substantial amount of geraniol (**2**) was formed, although no quantitative data were reported in that study. Their results indicate that breakage of both the C1−O and the glycosidic bond may have occurred at the higher temperature. Strauss and Williams (1983) noted that when glucopyranoside (**2a**) was hydrolyzed (for 30 min at an unspecified temperature), the proportion of total monoterpene ethers to alcohols was approximately equal to the molar ratio of ethanol to water present in the reaction mixture. No ethyl ethers, in particular geranyl ethyl ether, were detected in our study.

From the amounts of linalool (**13**) plus α-terpineol (**14**) formed at 80 °C after 1 h from geraniol (**2**) or its glucoside (**2a**), the aglycon geraniol (**2**) reacts at a rate ∼10 times faster than its glucoside (**2a**) (Tables 1 and 2). At the lower temperature (50 °C), the differences were even greater. These results are in good agreement with the recently published results of Ohta et al. (1991), who reported that the conversion of geraniol-β-D-glucoside (**2a**) to linalool (**13**) at 100 °C was ∼8 times slower than the corresponding hydrolytic conversion of geraniol (**2**). The reactivity of the glucoside (**2a**) is contrary to the belief expressed earlier (Williams et al., 1982, 1989) that glycosides of allylic alcohols (e.g., geraniol) form the allylic cation more readily than do the corresponding aglycons and that geraniol (**2**) is a major product of the hydrolysis of geranyl glucoside (**2a**).

Hydrolysis of Alcohols and Glycoconjugates Related to β-Damascenone (1) Formation. Ohloff et al. (1973) have shown that the acetylenic triol (**26**) gave β-damascenone (**1**) and 3-hydroxy-β-damascone (**6**) upon strong acid hydrolysis and suggested that the enyne diol (**4**) was an intermediate in this conversion. Subsequently, the enyne diol (**4**) was confirmed as a grape/wine constituent both in free and in glycoconjugated forms (Sefton et al., 1989; Baderschneider et al., 1997). Preliminary studies by us showed that both the aglycon (**4**) and the glucoside (**4a**) give β-damascenone (**1**) and 3-hydroxy-β-damascone (**6**) on mild hydrolysis (Skouroumounis et al., 1993). In the study reported here, the role of glycoconjugation in modifying the hydrolytic behavior of the enyne diol (**4**) was first examined by studying the hydrolysis of the model propargyl alcohol (**3**) and the corresponding glucoside (**3a**). The results are shown in Table 3.

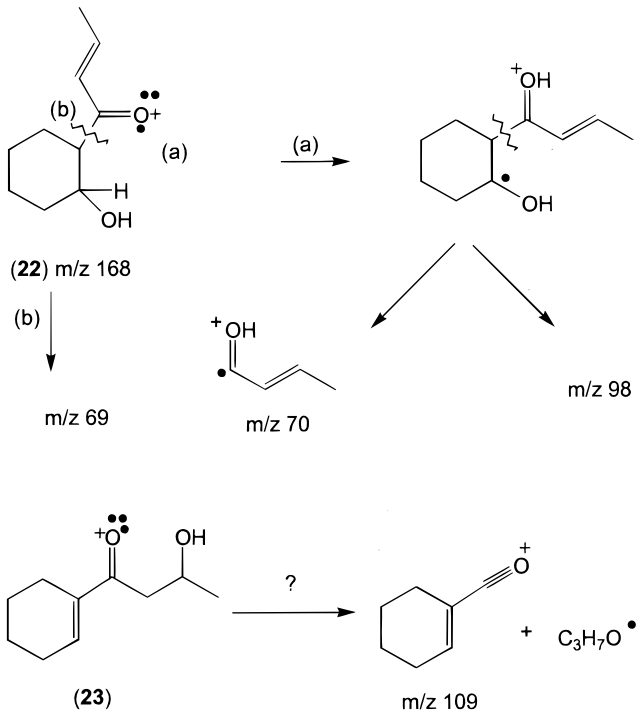
At pH 3 and 100 °C the reactivity of compound **3** was low. After 46 h, ∼26% of compound **3** had reacted to give two major products, a ketone (**21**), formally derived via a Meyer−Schuster rearrangement (Swaminathan and Narayanan, 1971; Edens et al., 1977), and a second product, tentatively identified from its mass spectrum as the hydrate (**22**). The mass spectrum of compound **22** included a molecular ion at *m/z* 168 and no characteristic *m/z* 109 ion peak via loss of 59 ($C_3H_7O^{\bullet}$), expected for the C9 hydroxy ketone (**23**), but it did have a base peak of 98, a strong *m/z* 70 (37%), and a significant *m/z* 69 (9%) ion (Scheme 1).

Table 3. Hydrolysis of Aglycon (3) and β-D-Glucoside (3a) at pH 3 and 100 °C

	aglycon (**3**)			β-D-glucoside (**3a**)			
	product (% yield)				product (% yield)		
time	**3**	**21**	**22**		**3**	**21**	**22**
4 h	98.5	1	0.5	4 h	0.9	0.5	trace
8 h	94.8	3.9	1.3	8 h	1.9	1.4	0.2
24 h	83.5	13.6	2.9	24 h	5.3	5.6	0.7
46 h	73.7	21.5	4.8	46 h	5.9	7.4	0.9

[a] Coefficients of variation for all analyses were <12%.

Scheme 1. Interpretation of the Mass Spectrum of Compound 22



The hydrolysis of the C9 glucoside (**3a**) was found to be slower than that of the aglycon (**3**). After 46 h, the products were detected in 14% yield only, compared to 26% for the aglycon. The major products were the aglycon (**3**), ketone (**21**), and β-hydroxyketone (**22**). It is uncertain whether the formation of the aglycon (**3**) arose via hydrolysis of the glycosidic or the ether linkage. Thus, the magnitude of the reduction in the rate of breakage of the ether bond, associated with glycosylation of aglycon (**3**), could not be determined. However, the formation of rearrangement products **21** and **22** was ∼3times faster with the aglycon (**3**) than with the glucoside (**3a**) (Table 3). The hydrolytic studies clearly indicate that the model acetylenic alcohol (**3**) and its β-glucoside (**3a**) undergo relatively slow hydrolysis and that the propargylic glucoside reacts more slowly than its corresponding aglycon.

Table 4. Products of the Hydrolysis of Enynediol (4) and Its C-9 Glucopyranoside (4a) at pH 3

	aglycon (**4**)					β-D-glucoside (**4a**)[a]			
		product (% yield)					product (% yield)		
	% conversion	**1**	**6**	**25**	**24**		**1**	**6**	**4**
100 °C						100 °C			
4 h	23	2.8	20	–	–	4 h	0.6	2.1	1.9
1 day	81	10	71	trace	trace				
2 days	98	14	83	trace	trace	2 days	5.0	33	1.8
50 °C						50 °C			
1 day	2	0.1	1.8	–	–				
7 days	13	0.8	12	–	–	7 days	0.14	1.6	1.1
28 days	44	2.3	41	–	–	28 days	1.0	11	3.8
20 °C									
28 days	2.6	trace	2.6	–	–				
90 days	6.4	0.2	6.2	–	–				
1 year	22	0.9	21	trace	trace				
6.3 years[b]	82	2.6	61	1.8	12				

[a] Some of these data have been reported previously Skouroumounis (1993). –, not detected. Coefficients of variation for all analyses were <12%. [b] Trace quantities of ethyl ether of compounds **24** and **25** at C-9 were also tentatively identified.

The yields of products of the hydrolyses of the enyne diol (**4**) and its glucoside (**4a**) under various conditions are shown in Table 4.

Hydrolysis of the enyne diol (**4**) at pH 3 and 100 °C gave β-damascenone (**1**) and 3-hydroxy-β-damascone (**6**) as the major products. The latter is not converted to the former under these conditions (see below), and so at any given pH and reaction temperature the ratio of compound **1** to compound **6** remained constant, regardless of reaction time. Small quantities of the hydrates **24** and **25** (tentatively identified only) were observed with prolonged hydrolysis. This indicates that, in the absence of significant generation of β-damascenone (**1**) from one or more precursors during aging, β-damascenone (**1**) [and 3-hydroxy-β-damascone (**6**)] already present in wine can decrease in concentration through formation of this hydrate.

β-Damascenone (**1**) and 3-hydroxy-β-damascone (**6**) were formed ~8 times more slowly from the glucoside (**4a**) than from the aglycon (**4**) (Table 4). However, at the lower temperature (50 °C) the proportion of β-damascenone (**1**) formed was greater from the glucoside (**4a**) than from the aglycon (**4**). It appears that by slowing the acid-catalyzed ionization of the propargyl alcohol function, the presence of the glycopyranosyl moiety promotes other competing transformations within the molecule. The side chain of **4a** can react either directly to give Meyer–Schuster rearrangement products or indirectly via the aglycon (**4**).

As previously reported (Skouroumounis et al., 1993), the ratio of β-damascenone (**1**):3-hydroxy-β-damascone (**6**) in the products of the hydrolysis of compounds **4** or **4a** is dependent on the reaction temperature. The ratio is a reflection of the selectivity of ionization exhibited at the different temperatures, with the proportion of β-damascenone (**1**) in the products from both the aglycon (**4**) and the glycoside (**4a**) being greater at a higher temperature. Thus, heating wine (or juice) is not only likely to accelerate the generation of β-damascenone (**1**) but could ultimately lead to a higher yield of this compound than could be obtained by natural wine aging.

The reactivity of compounds **4** and **4a** is significantly greater than that of the analogues **3** and **3a**, and this is attributed to the extra methyl substituents which can stabilize incipient carbocations via the inductive effect.

Hydrolysis of 3,5,5-Trimethylcyclo-3-hexen-1-ol (5), 3-Hydroxy-β-damascone (6), and Their β-D-Glucopyranosides (5a and 6a). It has been suggested that 3-hydroxy-β-damascone (**6**) acts as a precursor of β-damascenone (**1**) in nature (Ohloff, 1978). However, Ohloff et al. (1973) have also demonstrated that 3-hydroxy-β-damascone (**6**) does not give β-damascenone (**1**) under strongly acidic conditions but rather products derived by Nazarov type cyclization (Rouvier and Santelli, 1983). Nevertheless, nothing is known about the behavior of the corresponding glucoside (**6a**) under mild acid conditions. Before studying this hydrolysis, we examined the hydrolysis of 3,5,5-trimethylcyclo-3-hexen-1-ol (**5**) and its glucoside (**5a**), as a model for homoallylic cyclohexenols at wine pH, as these functional groups are common to many C13 norisoprenoids. Recently, the glucoside (**5a**) has been identified as a natural product in saffron (Straubinger et al., 1998).

Table 5. Hydrolysis of the Aglycon (5), Glucoside (5a), and Glucoside (6a) at Various pH, Time, and Temperature Conditions

	yield[a] (%)					
conditions	**27a–d**	**5**	**6**	**28**	**29**	**30**
aglycon (**5**)						
pH 3, 100 °C, 24 h	+, +, +, +	83		1.5	6.5	1.0
pH 1, 50 °C, 24 h	+, +, +, –	79		2.0	8.4	1.0
pH 1, 80 °C, 24 h	14, 15, 8.0, 11	22		1.0	12	2.0
pH 1, 100 °C, 8 h	38, 13, 12, 19	3.2		2.1	5.9	2.7
glucoside (**5a**)						
pH 3, 100 °C, 24 h	– – – –	13		–	–	–
pH 1, 50 °C, 24 h	– – – –	8.5		–	–	–
pH 1, 80 °C, 24 h	+, +, +, +	24		–	+	–
pH 1, 100 °C, 8 h	39, 10, 12, 19	10		–	4.5	–
glucoside (**6a**)						
pH 3, 100 °C, 24 h			16			
pH 1.1, 100 °C, 8 h			35			

[a] –, not detected; +, trace amount, <0.1%. Coefficients of variation for all analyses were <12%.

At pH 3 the aglycon (**5**) was relatively unreactive, and even after 24 h at 100 °C, it was found that only 17% had reacted. The main products, identified from their mass spectra, were trace amounts of the isomeric dienes of undetermined structure (**27a–d**), the isomeric alcohol (**28**), and small quantities of oxidation products **29** and **30** (Table 5). The β-glucoside (**5a**) gave ~13% of the aglycon (**5**) under these conditions. Under more forcing conditions (pH 1.0), various products were observed (Table 5).

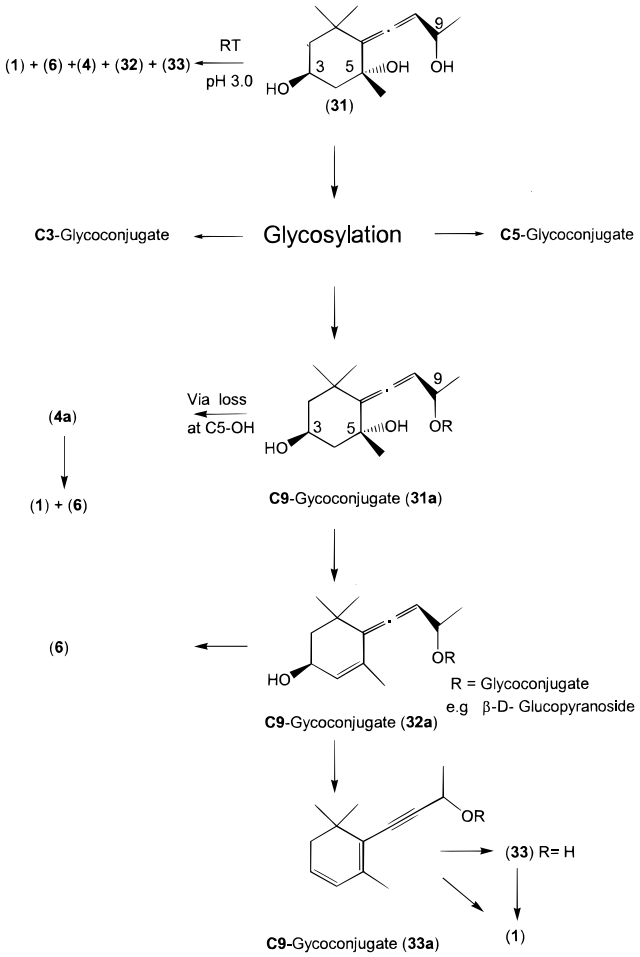
At 80 °C, 24 h, the aglycon (**5**) underwent dehydration, yielding a total of 48% diene formation, whereas the glycoside (**5a**) yielded trace amounts of dienes and aglycon (**5**) (24%).

At pH 1 and 100 °C, virtually all of the aglycon (**5**) had reacted after 8 h with multiple product formation. Only 3.2% of the aglycon remained, and **27a**−**d** accounted for 82% of the products. The glycoside (**5a**), under the same conditions, yielded 80% dienes (**27a**−**d**) and 10% aglycon (**5**). It is evident that at the lower temperatures there are differences between the reactivity of the glycoside and aglycon but at the highest temperature (100 °C) the products were formed in similar yields after 8 h, with dehydration products predominant.

Although these results indicate that simple homoallylic cyclohexenols and their glucosides are likely to have low reactivity at wine pH, it is conceivable that the additional conjugation with the enone side chain of *β*-D-glucopyranoside (**6a**) could enhance the reactivity of the homoallylic glycosidic function. However, when the hydrolysis of *β*-D-glucopyranoside (**6a**) was carried out at pH 3.0 and 100 °C for 24 h, 16% of aglycon, 3-hydroxy-*β*-damascone (**6**), was obtained but no trace of *β*-damascenone (**1**) could be detected. At pH 1.1 and 100 °C for 8 h, only 3-hydroxy-*β*-damascone (**6**) was obtained in 35% yield (Table 5). No elimination products were seen with the hydrolysis of the glycoside (**6a**), even under forcing conditions. The reason for these differences is unclear. It is possible that the side chain is easily enolized and that this favors the formation of glycoconjugates of Nazarov cyclization products, which are known to prevail under even more forcing conditions (Rouvier and Santelli, 1983). To check this hypothesis, hydrolysis of 3-hydroxy-*β*-damascone (**6**) was carried out in deuterated solvents. After 4 h at 100 °C and pH 3.0, 3-hydroxy-*β*-damascone (**6**) was found to be stable and there was no deuterium incorporation. However, at pH 1.0 and 100 °C for 80 min, Nazarov cyclization products were evident in trace amounts and the 3-hydroxy-*β*-damascone (**6**) incorporated deuterium, albeit only in the side chain methyl and not in the ring. This was demonstrated by high-field ^{1}H NMR spectroscopy, which showed the collapse of both the side chain vinyl methyl doublet and the vinyl proton at C8 to complex multiplets. The C4 multiplets remained unchanged. This suggests that the enone side chain is rotated out of conjugation with the ring double bond, presumably to relieve steric interaction with the geminal dimethyl groups. Thus, the hydrolytic behavior of 3-hydroxy-*β*-damascone glucoside (**6a**) was unlikely to be influenced by enolization in the cyclohexenol ring.

Implications for *β*-Damascenone (1) Formation from the Allenic Triol (31). The multifunctional allenic triol (**31**) (Scheme 2) is known to have a half-life of <24 h at pH 3 and room temperature (Skouroumounis et al., 1992, 1993), yet a glycoconjugate has been islolated from *Lyium halimifolium* Mil. (Naf et al., 1990) at the natural plant pH of 4.0 as well as from apples *Malus sylvestris* Mill. cv. Jonathan (Winterhalter et al., 1993). The allenic triol (**31**) has also been observed in enzyme hydrolysates of glycosidic fractions obtained from Merlot grapes (Sefton, 1998). The allenic triol (**31**) has been postulated as the natural precursor to *β*-damascenone (**1**) (Ohloff et al., 1973; Isoe et al., 1973), and recent studies in our laboratories (Skouroumounis et al., 1992) have shown the allenic triol (**31**) does give *β*-damascenone (**1**) in small amounts, <1%, after 24 h at 24 °C and pH 3.0. The two major products, however, were 3-hydroxy-*β*-damascone (**6**) and enynediol (**4**). Trace amounts of the allenic diol (**32**) and the dienealkyne (**33**) were tentatively identified as intermediates in this transformation (Skouroumounis et al., 1992).

Scheme 2. Hypothetical Pathways for the Decomposition of a C-9 Glycoconjugate of the Allenic Triol 31



It was evident from these studies that the three end products of allene triol (**31**) hydrolysis were formed from several competing pathways. Glycosylation (which is thought to be the terminal step in the biotransformations of secondary metabolites) is likely to influence the relative importance of these pathways (e.g., Scheme 2). Thus, the tentatively identified bifunctional intermediate (**32**) might eliminate a hydroxyl function, under acid conditions, to form either the C3 or C9 carbocation (megastigmane numbering; Aasen et al., 1972), the former leading to the dienealkyne (**33**) and ultimately to *β*-damascenone (**1**), and the latter process leading mostly to 3-hydroxy-*β*-damascone (**6**) (Skouroumounis et al., 1992). If either the C3 or C9 position is stabilized by glycosylation, then ionization of the underivatized hydroxyl can be favored. In the case of C9 glycosylation, this would be expected to enhance *β*-damascenone (**1**) formation.

Conclusion. Allylic, propargylic, and homoallylic alcohols are more reactive than their corresponding *β*-D-glucopyranosides. Although the rates of hydrolysis of such glucosides are significantly lower than for the aglycons (possibly orders of magnitudes lower in some cases), this may nevertheless have an important impact on the direction of flavor release. In the case of floral grape varieties, where geraniol (**2**) is concerned, glycosylation is a process that lowers the flavor of the grape juice/wine, not only because geraniol (**2**) itself is an

important flavorant in some wines but because the rate of formation of other flavor compounds from geraniol (**2**) is also reduced. However, when flavor compounds in wines are formed from polyfunctional aglycons in competition with flavorless byproducts by hydrolytic reactions, then glycoconjugation through its influence of the reactivity of alcoholic functional groups can influence the fate of the aglycon and can either enhance or suppress the formation of flavor.

ACKNOWLEDGMENT

We thank Drs. R. A. Massy Westropp and P. J. Williams and Professors P. Winterhalter and P. Høj for their advice and encouragement.

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Received for review May 12, 1999. Revised manuscript received January 21, 2000. Accepted January 31, 2000. We thank the Grape and Wine Research and Development Corp. for funding this research.

JF9904970