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Rationalizing the Formation of Damascenone: Synthesis and Hydrolysis of Damascenone Precursors and Their Analogues, in both Aglycone and Glycoconjugate Forms

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Storage of megastigma-4,6,7-trien-3,9-diol (**5**), and megastigma-3,4-dien-7-yn-9-ol (**6**) in aqueous ethanol solution at pH 3.0 and 3.2 gave exclusively damascenone (**1**) and damascenone adducts at room temperature. The diol (**5**) had half-lives for the conversion of 32 and 48 h at pH 3.0 and pH 3.2, respectively. The acetylenic alcohol (**6**) had half-lives of 40 and 65 h at the same pH levels. In order to study the reactivity of the C-9 hydroxyl function in **5** and in the previously investigated allenic triol **2**, two model compounds, megastigma-4,6,7-trien-9-ol (**7**) and megastigma-6,7-dien-9-ol (**8**) were synthesized. No 1,3-transposition of oxygen to form analogues of damascenone was observed when **7** and **8** were subjected to mild acidic conditions. Such transposition takes place only with highly conjugated acetylenic precursors such as **6** or tertiary allenic alcohols such as **2**. The placement of glucose at C-3 of **5** and at C-9 of **6** gave the glycosides **9** and **10**, respectively. The effect of such glucoconjugation was to increase the observed half-lives by a factor of only 1.6−1.7 for the allenic glucoside **9**, and by 2.1−2.2 for the acetylenic glucoside **10**. These studies indicate that the effect of glycosylation on damascenone formation is probably not important on the time scale of wine making and maturation.

KEYWORDS: Damascenone; allenic alcohols; acetylenic alcohols; glycoconjugate; hydrolysis; norisoprenoid; carotenoid metabolites; oxygen transposition.

INTRODUCTION

β-Damascenone (more recently referred to as simply damascenone, **1**) is one of the most important natural aroma compounds known; it has been identified in many different types of plant material and is also one of the mainstays of the international perfume industry (*1*). Thirteen-carbon secondary metabolites with the megastigmane skeleton, including damascenone (**1**), are widely found in plants and plant products (*2*−*6*). Most of these contain oxygen at the C-9 position, which is usually presumed to have resulted directly from oxidative cleavage of a carotenoid precursor. Simple carotenoid degradation alone, however, cannot directly generate 7-oxygenated megastigmanes such as damascenone (**1**). As **1** can be formed by mild acid hydrolysis of crude plant isolates, much research has focused on chemical mechanisms for transposition of oxygen from C-9 to C-7 to form damascenone, resulting in the identification of the allenic triol **2** as a damascenone precursor (*7*). At room temperature and under mild acid conditions, **2** is rapidly converted to 3-hydroxydamascone (**3**) and the enyne diol **4**, as major products, along with small amounts of damascenone (**1**) (**Figure 1A**) (*7*). The enyne diol **4** was also found to produce both **1** and **3**, but at a rate several orders of magnitude lower than that observed for **2** (*5*). The lower reactivity of **4** was considered to be consistent with the observation by Olsson et al. (*8*) that, under acidic conditions, α-hydroxy allenes rearrange much faster than do α-hydroxy acetylenes.

More recently, both the dienyne species **6** (*9*) and the allenic diol **5** (*10*) were shown to be intermediates in the formation of damascenone from **2**, and the former was observed as an intermediate in the hydrolysis of the latter (**Figure 1B**). No trace of 3-hydroxydamascone (**3**) was found from hydrolysis of either

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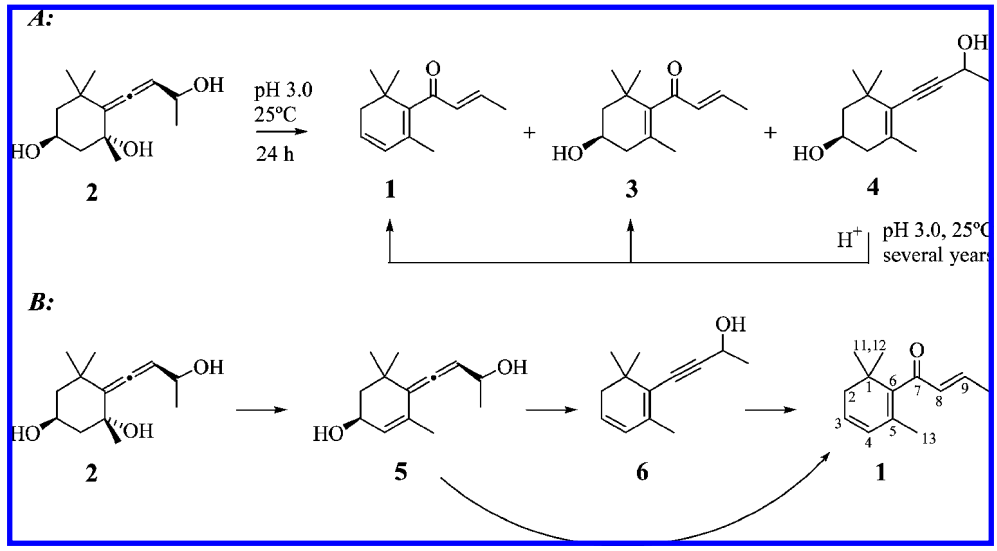

Figure 1. **A**: Major hydrolysis products of allenic triol **2**. **B**: Route of formation of damascenone **1** from allene triol **2**.

5 or **6**. This indicated the possibility that the formation of **3** from **2** might proceed directly, via loss of the C-9 α-allenic hydroxyl in **2**. Puglisi et al. (*10*) observed that, under the reaction conditions (pH 3.0, model wine), the diol **5** initially reacted exclusively at C-3, presumably because this generates the more stable cation. However, placement of a glycoconjugating unit on the C-3 hydroxyl group could conceivably reduce the reactivity at this site to the point where reaction at C-9 becomes competitive (*5*).

We report, here, a study of the rate of formation and yields of damascenone (**1**) from the aglycones **5** and **6**, and their corresponding glucoconjugates **9** and **10**. This was conducted in order to determine whether glycoconjugation had important effects on the rates of reaction of, and product distribution from, the aglycone forms. We have also studied the reactivity of the model compounds **7** and **8** in order to assess whether transformations of secondary α-hydroxy allenes can take place under physiological or mild food processing conditions when competing reactions are absent or slow.

MATERIALS AND METHODS

Materials. Chemicals were purchased from Sigma-Aldrich. (*S*)-Phorenol (**16**) (ee >96%) was provided by Dr. K. Puntener, Hoffmann-La Roche International. All solvents used were pesticide grade from OmniSolv (Darmstadt, Germany). X4 is a mixed hydrocarbon solvent, with *n*-hexane as the major component. All organic solvent solutions were dried over anhydrous sodium sulfate before being filtered. pH measurements were made with a EcoScan pH 5/6 m (Eutech Instruments, Singapore), which was calibrated before use. Column chromatography was performed using silica gel 60 (230−400 mesh) from Merck. Optical rotations were measured on a Polaar 21 polarimeter operating at 20 °C. Routine ^{1}H and ^{13}C NMR spectra were recorded (in $CDCl_3$) with a Varian Gemini spectrometer at operating frequencies of 300 and 75.5 MHz, respectively. All compounds gave spectroscopic data that were consistent with the expected structures. Mass spectra were recorded on a Hewlett-Packard (HP) 6890 gas chromatograph fitted with a liquid HP 6890 series injector and coupled to a HP 5973 mass spectrometer, with the instrument set up as described by Janusz et al. (*11*); specifically, the oven temperature was started at 80 °C, held at this temperature for 1 min, increased to 170 at 4 °C/min, then to 250 at 50 °C/min, and held at this upper temperature for 10 min. The injector was held at 220 °C and the transfer line at 260 °C. Buffer solutions were prepared by saturating a 10% ethanol solution with potassium hydrogen tartrate and adding 10% tartaric acid solution until reaching the required value. Damascenone was quantified by the method reported in Daniel et al. (*12*), which utilizes GC/MS and d_4-damascenone as internal standard.

Methods. *(9R)-9-Hydroxymegastigma-3,5-dien-7-yne (**6**).* To a solution of (*R*)-but-3-yn-2-ol (2.1 g, 0.03 mol) in cold (−78 °C) ether (40 mL) was added *n*-BuLi (2.5 M in hexanes, 23 mL, 0.058 mol) dropwise. The reaction mixture was allowed to warm to room temperature before enone **20** (*9*) (2.78 g, 0.02 mol) was added dropwise. The reaction was stirred at room temperature for 2 days before being quenched with saturated NH_4Cl. The organic layer was diluted with ethyl acetate and washed with brine, before being dried (Na_2SO_4) and purified by silica column chromatography to give (9*R*)-6,9-dihydroxymegastigm-4-en-7-yne (3.63 g, 87%). To this was added pyridine (4.15 g, 52.5 mmol, 4.24 mL) and acetic anhydride (3.57 g, 35 mmol, 3.3 mL). The mixture was heated at reflux overnight, cooled, and diluted with ethyl acetate. The organic layer was washed with 10% HCl solution, aqueous Na_2SO_4 and brine before being dried and concentrated *in vacuo*. The crude material was purified by column chromatography (15% ethyl acetate in hexane) to produce (9*R*)-6-hydroxy-9-acetoxymegastigm-4-en-7-yne (3.2 g).

To a predried flask containing P_2O_5 (16 g) and celite (8 g), under an inert atmosphere, was added toluene (50 mL) and pyridine (10 mL). The mixture was stirred to form a slurry, and a solution of the previously prepared acetate (1.0 g, 4.8 mmol) in toluene (5 mL) was then added. The mixture was stirred at reflux for 2 days, cooled, filtered, and washed with diethyl ether followed by ethyl acetate. The residue was concentrated in vacuo and purified by column chromatography to yield (9*R*)-9-acetoxymegastigma-3,5-dien-7-yne (0.14 g, 22%).

To a 50% aqueous ethanol solution (2 mL) was added KOH (0.015 g, 0.14 mmol) and (9*R*)-9-acetoxymegastigma-3,5-dien-7-yne (0.03 g, 0.13 mmol). The mixture was stirred for 2 h at room temperature, and the residue was then extracted with dichloromethane. The organic layer was washed with water and brine, dried (Na_2SO_4) and concentrated *in vacuo* to yield (9*R*)-(**6**) (0.0247 g, 99%).

*(9S)-9-Hydroxymegastigma-3,5-dien-7-yne (**6**).* The (9*S*) enantiomer of **6** was prepared in a manner identical to that described above for the (9*R*) enantiomer, with the only difference being the use of (*S*)-but-3-yn-2-ol to introduce the stereochemistry on the side-chain. Except for the sign of the optical rotation measured, the pure (9*S*)-enantiomer was indistinguishable from the (9*R*)-enantiomer.

*(9R)-9-O-(β-D-glucopyranosyl)-megastigma-3,5-dien-7-yne (**10**).* To a solution of (9*R*)-**6** (0.12 g, 0.65 mmol) in dry dichloromethane (3 mL) was added silver triflate (0.03 g, 1.1 mmol, 1.6 eq), *S*-collidine (0.14 mL, 1.1 mmol, 1.6 eq) and α-bromo-2,3,4,6-tetrapivaloylglucose **19** (0.57 g, 0.98 mmol, 1.5 eq). The reaction was stirred overnight at room temperature with the exclusion of light. The reaction was quenched with $NaHCO_3$ solution, and the organic layer was diluted with dichloromethane. The organic layer was washed with brine, dried

($MgSO_4$), and concentrated *in vacuo* to yield 0.66 g of a brown toffee-like substance. The crude material was purified by column chromatography (5% ethyl acetate in X4) to yield 0.32 g (72%) of the tetrapivaloyl-protected glucoside.

To a solution of a portion of this protected glucoside (0.02 g, 0.36 mmol) in methanol (2 mL) was added sodium methoxide (4 eq) in methanol (2 mL). The mixture was stirred at room temperature overnight (monitored by TLC) and purified using column chromatography (Merck silica 60 HF_{254}) (10% MeOH in dichloromethane.) to yield pure material (9*R*)-(**10**) (9.7 mg, 75%).

*(9S)-9-O-(β-D-glucopyranosyl)-megastigma-3,5-dien-7-yne (***10***).* (9*S*)-(**6**) was converted into the corresponding glycoside by a method identical to that described above for the (9*R*) enantiomer.

*(9R)-9-Hydroxymegastigma-4,6,7-triene (***7***).* (*R*)-But-3-yn-2-ol (762 mg, 0.01 mol) was added to ketone **20** (500 mg, 3.6 mmol) by a procedure identical to that described above for compound **6**. The product was recrystallized from X4 to yield diol (9*R*)-**21** as a white solid (485 mg, 64%). To a solution of (9*R*)-**21** (465 mg, 2.24 mmol) in dry ether (25 mL) was added $LiAlH_4$ (594 mg, 15.6 mmol) at 0 °C. The mixture was heated under reflux for 5 h, then quenched with acetone, diluted (ether), washed (10% NaOH solution, brine), dried (Na_2SO_4), and the solvent evaporated. The product was purified by column chromatography (10% EtOAc/X4) to give a colorless oil (313 mg, 73%).

(9S)-9-Hydroxymegastigma-4,6,7-triene (7). The (9*S*) analogues of **7** were prepared by methods identical to those described above using (*S*)-But-3-yn-2-ol.

*(9R)-9-Hydroxymegastigma-6,7-diene (***8***).* (*R*)-But-3-yn-2-ol (151 mg, 2.16 mmol) in ether (10 mL) at −78 °C was treated with *n*-BuLi (2.5 M in hexanes, 1.7 mL, 4.25 mmol) and the reaction stirred for 1 h at room temperature. The solution was recooled to −78 °C and added dropwise to a solution of **22** (100 mg, 0.71 mmol) in ether (5 mL) at −78 °C. The mixture was warmed to room temperature and stirred for 16 h, then quenched with NH_4Cl, washed with water and brine, dried (Na_2SO_4), and the solvent evaporated. The product was purified by column chromatography to yield (9*R*)-**23** as a colorless oil (127 mg, 84%). To a solution of alkyne (9*R*)-**23** (127 mg, 0.61 mmol) in Et_2O (10 mL) at 0 °C was added $LiAlH_4$ (161 mg, 4.23 mmol). The reaction was heated under reflux for 5 h, then quenched with acetone, diluted with Et_2O, washed with an aqueous solution of 10% NaOH, brine, dried (Na_2SO_4), and the solvent evaporated. Purification by column chromatography (10% EtOAc/X4) yielded 33 mg (28%) of (9*R*)-**8** as a colorless oil.

*(9S)-9-Hydroxymegastigma-6,7-diene (***8***).* The (9*S*) analogues of **8** were prepared by methods identical to those described above using (*S*)-but-3-yn-2-ol.

General Hydrolysis Procedure for Allenes **7** *and* **8.** Each of (9*R*)-**7**, (9*S*)-**7**, (9*R*)-**8**, and (9*S*)-**8** (50 mg/L) was dissolved in model wine (5 mL, pH 3.0) with naphthalene (50 mg/L) as internal standard and sealed in glass ampoules under a nitrogen atmosphere. Triplicate ampoules were heated at 25 °C for 12, 24, 72, and 168 h or at 45 °C for 12, 24, and 72 h. The contents were then extracted with pentane/EtOAc (2:1) and analyzed by GC-MS. Chiral GC was conducted using a cyclosil-B column, 30 m × 0.25 mm × 0.25 μm.

*(9S)-9-Ethoxymegastigma-6,7-diene (***11***).* KH (60 wt. % in mineral oil, 20 mg, 0.29 mmol) was washed with X4 and added to a stirred solution of (9*S*)-**8** (25 mg, 0.13 mmol) in DMF (2 mL) at 0 °C. The mixture was stirred cold for 30 min, then ethyl iodide (200 μL, 2.5 mmol) was added and the mixture warmed to room temperature and stirred for 1 h. The reaction was diluted with Et_2O, dried, and the solvent evaporated to give a crude mixture of the product, which was purified by column chromatography (5% EtOAc/X4) to yield 17 mg (59%) of a colorless oil.

*(4S)-2,6,6-Trimethyl-4-O-(2′,3′,4′,6′-tetrapivaloyl-β-D-glucopyranosyl)-cyclohex-2-en-1-one (***17***).* To a solution of alcohol (*S*)-**16** (100 mg, 0.65 mmol) in dry dichloromethane (2 mL) was added silver triflate (133 mg, 0.52 mmol), s-collidine (138 μL, 1.04 mmol), and **19** (540 mg, 0.93 mmol). A second portion of silver triflate (133 mg, 0.52 mmol) was added and the mixture diluted with CH_2Cl_2 (3 mL), then stirred at room temperature in the absence of light for 16 h. The reaction was quenched with a saturated solution of sodium bicarbonate, washed with brine, dried (Na_2SO_4), and the solvent evaporated. The product was purified by column chromatography (10% EtOAc/X4) to yield 262 mg (62%) of a white crystalline compound (mpt 163−165 °C).

*(3S,9R)-3-(O-β-D-Glucopyranosyl)-9-hydroxymegastigma-4,6,7-triene (***9***).* (*R*)-But-3-yn-2-ol (141 mg, 2.0 mmol) in anhydrous ether (10 mL) at −78 °C was treated with *n*-BuLi (2.5 M, 1.61 mL, 4.02 mmol) and the mixture stirred at room temperature for 1 h. It was then recooled to −50 °C and ketone **17** (262 mg, 4.0 mmol) in ether (10 mL) added dropwise. The reaction was warmed to room temperature and stirred for 2 days, then quenched with a saturated solution of NH_4Cl, diluted with ether, washed with brine, dried (Na_2SO_4), and the solvent evaporated. The product was purified by column chromatography (40% EtOAc/X4) to yield 151 mg (52%) of (3*S*,9*R*)-**18** as a white solid. To a solution of this alkyne **18** (151 mg, 0.21 mmol) in anhydrous ether (10 mL) at 0 °C was added $LiAlH_4$ (56 mg, 1.48 mmol). The mixture was heated under reflux for 5 h, quenched with EtOAc and a small amount of water, and the solvent removed. The residue was dissolved in MeOH, the solution filtered, and solvent removed. The product was purified by column chromatography (15% MeOH/CH_2Cl_2) to yield 40 mg (52%) of a sticky solid.

General Hydrolysis Procedure for Aglycones **5**,**6** *and glucosides* **9**,**10.** Individual solutions of **5**, **6**, and **9** and both (9*R*)-**10** and (9*S*)-**10** (1 mg/L and 1.7 mg/L for aglycones and glycosides, respectively) in model wine (buffered 10% aqueous ethanol) at pH 3.0 and 3.2 were sealed in ampoules in triplicate and heated at 25 °C in a water bath. Ampoules were removed periodically, opened, and to 1 mL was added internal standard (d_4-damascenone), and the solution was extracted with a 2:1 mix pentane/ethyl acetate and subjected to GC-MS analysis (ZB-Wax, 30 m × 0.25 mm × 0.25 μm column) to determine the formation rates of β-damascenone. The damascenone content was quantified as described in Daniel et al. (*12*).

RESULTS

Synthesis of the Glucosides 9 and 10. The diol **5** (*10*) and both the (9*R*) and (9*S*) isomers of the alcohol **6** (*9*) were prepared as described previously. Glycosylation of each of the dienyne alcohols **6** proceeded smoothly using a modified Koenigs−Knorr procedure (*13*) to give both (9*R*)-**10** and (9*S*)-**10**. We chose to use the tetra-pivaloylated bromoglucose (**19**) as the reagent for the introduction of the carbohydrate unit as we have found, in keeping with earlier reports (*14*), that this species produces only the β-anomer. Synthesis of the C-3 glycoside of allene **5** was not so straightforward; attempts to introduce the glucose unit at the C-3 position in **5** itself proved futile, with the aglycone being too unreactive under neutral conditions and prone to undergoing side reactions under acidic conditions. The desired glycoside was eventually prepared successfully via the method shown in **Figure 3**. Glucosylation of (*S*)-phorenol (*S*)-**16** (*15*) was successfully achieved (*16*) in 62% yield. Addition of (2*R*)-3-butyn-2-ol proceeded smoothly, with no evidence of addition of the acetylide species across the pivaloyl carbonyls. Finally, reduction with LAH accomplished both rearrangement of the side chain, as well as deprotection of the glycoside unit to give **9**.

Synthesis of the Allenes 7 and 8. Two forms of the target compound, **7**, differing in their stereochemistry at C-9, (**Figure 4**) were prepared by addition of the dilithio derivative of either (2*R*)- or (2*S*)-3-butyn-2-ol to 2,6,6-trimethylcyclohex-2-enone (**20**) (*9*) followed by reaction with LAH to give **7**, whose spectroscopic data were in accord with those previously published (*17*). In each case, the product was a pair of diastereomers that differed in the stereochemistry at the allenic position, C-6. Separation of the diastereomers was not considered to be crucial and was not attempted in either case. The second target compound, (**8**, **Figure 4**) was obtained, in an analogous manner, from 2,6,6-trimethylcyclohexanone (**22**). As was the case with **7**, the use of either (2*R*)- or (2*S*)-3-butyn-2-ol gave a mixture of diastereomers.

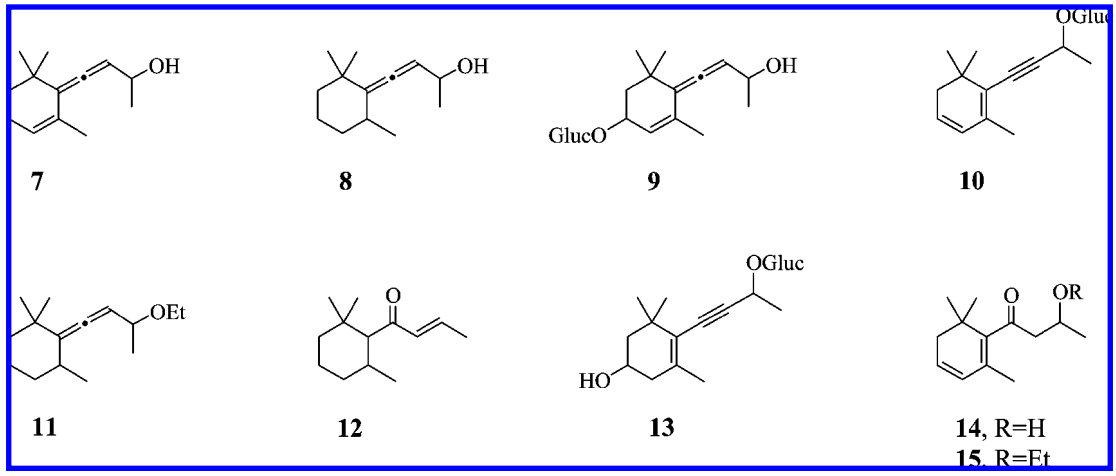

Figure 2. Structures of compounds studied.

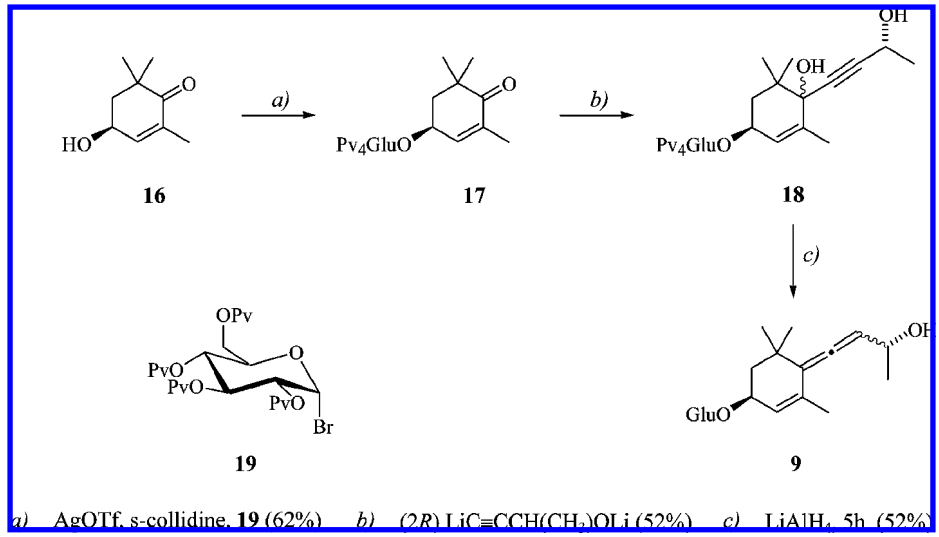

Figure 3. Synthesis of C-3 glycoside **9**.

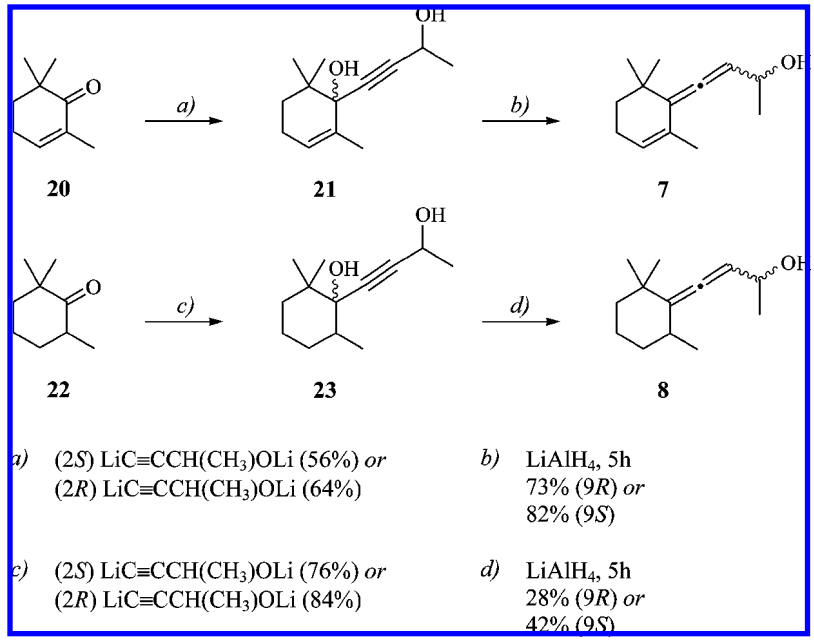

Figure 4. Synthesis of allenes **7** and **8**.

Hydrolysis of Aglycones 5 and 6 and Their Respective Glucosides 9 and 10. The hydrolyses of **5**, **6**, **9**, and **10** were all conducted in two model wine solutions (10% ethanol in water, pH 3.0 and pH 3.2) at 25 °C in sealed ampoules, which were removed at selected time intervals and analyzed for their damascenone content, by the procedure reported by Daniel et

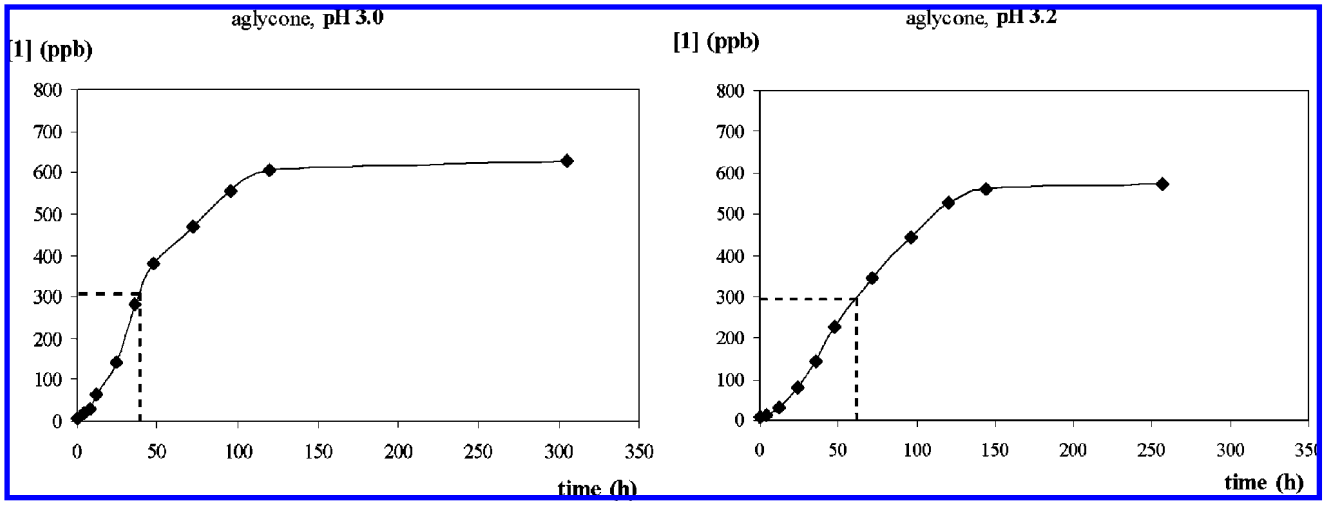

Figure 5. Formation of damascenone (**1**) over time during hydrolysis of aglycone **6** at pH 3.0 (left) and pH 3.2 (right). Both hydrolyses were conducted at 25 °C.

Table 1. Half-Lives and Glycoside/Aglycone Half-Life Ratios for the Formation of Damascenone (**1**) from Compounds **5**, **6**, **9**, and **10**

compound[a]	pH[b]	$t_{1/2}$[c,d]	ratio[e]
5	3.0	32	
	3.2	48	
9	3.0	50	~1.6
	3.2	80	~1.7
6	3.0	40	
	3.2	65	
(9*R*)-**10**	3.0	84	~2.1
	3.2	144	~2.2
(9*S*)-**10**	3.0	84	~2.1
	3.2	144	~2.2

[a] All hydrolyses were conducted at 25 °C and at starting concentrations of 1 mg/L (aglycones) or 1.7 mg/L (glycosides). [b] Solutions were buffered with potassium hydrogen tartrate and tartaric acid; [c] $t_{1/2}$ (hours) defined as the time taken for damascenone concentration to reach half its final value. [d] Half-lives determined by interpolation (e.g., **Figure 6**); errors estimated as ± 5%. [e] Defined as $t_{1/2\ glyc}/t_{1/2\ aglyc}$.

al. (*12*). In every case, the final products of reaction, observable by GC/MS, were damascenone (**1**) plus small amounts of both the C-9 hydrate **14** and the C-9 ethanol adduct **15**. In all hydrolysates, the final quantified amount of damascenone corresponded to approximately 70% of the theoretical maximum.

The evolution of damascenone from the propargyl alcohol **6** at both pH 3.0 and pH 3.2 over time is shown in **Figure 5**. From these data, the times for the concentration of damascenone to reach half of the maximum value ($t_{1/2}$) were determined as 40 and 65 h, respectively. Similar graphs were constructed for all hydrolyses. **Table 1** lists the $t_{1/2}$ values for the formation of damascenone from **6** and from the allenic precursor **5**, as well as the corresponding glycosides, **9** and (9*R*)- and (9*S*)-**10** at both pH values. These provide ratios ($t_{1/2glyc}/t_{1/2aglyc}$) of 1.6 at pH 3.0 and 1.7 at pH 3.2 for **9**:**5** and 2.1 at pH 3.0 and 2.2 at pH 3.2 for **10**:**6**. Both isomers of **10** were converted to damascenone at an identical rate.

Hydrolysis of the Allenes 7 and 8. The hydrolyses of **7** and **8** were conducted in model wine solution (10% ethanol in water, pH 3.0) at both 25 and 45 °C. The half-life of **7** at 25 °C was approximately 10 days. For both reaction temperatures, each pair of isomers of the hydroxy allene **7** produced product mixtures that were identical to one another by NMR and achiral phase GC. These comprised >90% of the total product and were identified as the isomeric 7,8-didehydrotheaspiranes (**24**, **Figure 6**) on the basis of their spectroscopic data (*17*). When the 7,8-didehydrotheaspiranes produced from the hydrolysis of (9*R*)-**7** were examined by chiral phase GC, only two isomers were observed, at retention times of 13.28 and 14.53 min. Similarly, the hydrolysis of (9*S*)-**7** produced two isomeric 7,8-didehydrotheaspiranes, with retention times of 14.38 and 14.87 min. This clearly shows that the C-9 hydroxyl did not undergo epimerization prior to cyclization.

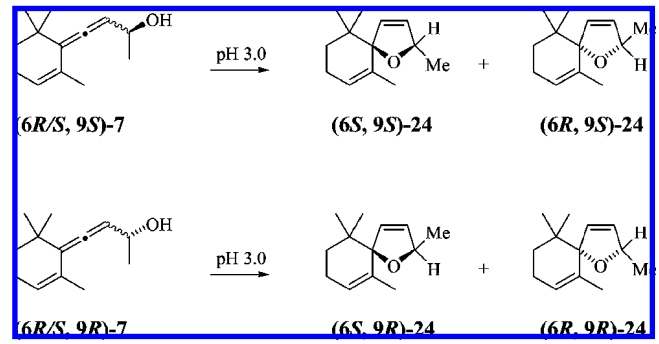

Figure 6. Stereochemical relationship between the starting allenes **7** and the 7,8-didehydrotheaspirane products obtained.

Hydrolysis of the hydroxy allene **8** proceeded more slowly; the substrate had a half-life of approximately 4 days at 45 °C and had undergone approximately 25% conversion after two weeks at 25 °C. A single product (albeit as mixtures of isomers that were not resolvable by chiral GC) was produced and was identified as the allenic ether **11**. This structural assignment was confirmed by synthesis of the allenic ether by treatment of **8** with potassium hydride followed by quenching with ethyl iodide. As was the case in the hydrolysis of **7**, no rearrangement to produce the enone side chain was observed, that is, compound **12** was not detected as a product.

DISCUSSION

We had earlier shown that the allene diol **5** and the acetylenic alcohol **6** were both intermediates in the hydrolytic conversion, at room temperature and pH 3.0, of allenic triol **2** into damascenone (**1**). They were not, however, intermediates in the formation of the two major hydrolysis products of **2**, that is, 3-hydroxydamascone (**3**) and the enyne diol **4** (**Figure 1A**) (*9, 10*). The alcohol **6** was also observed as an intermediate in the hydrolytic conversion of **5** into **1** (**Figure 1B**) (*10*). The

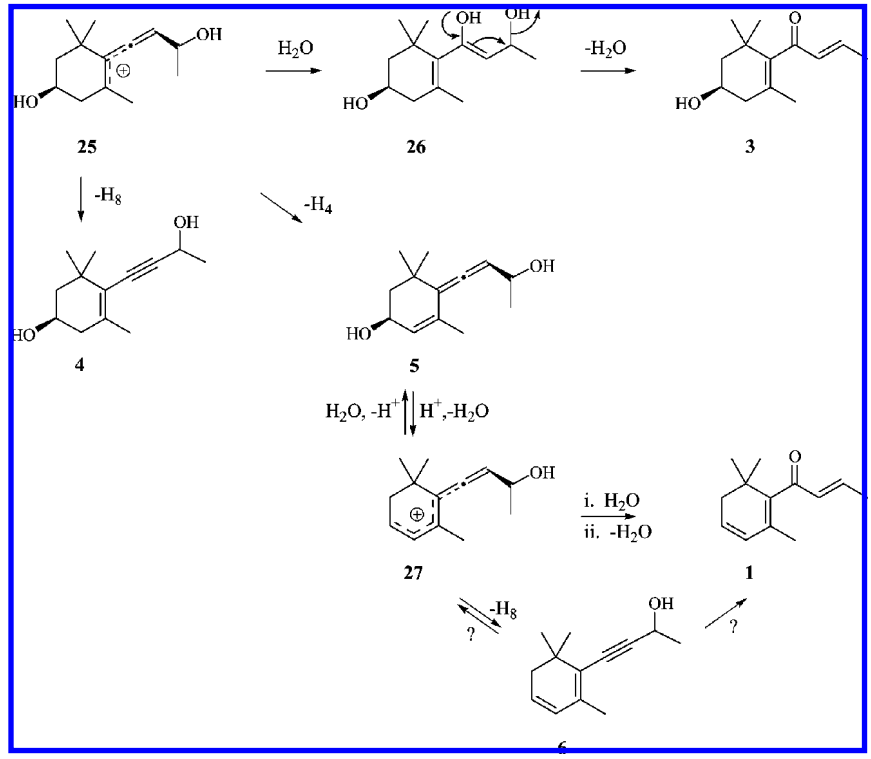

Figure 7. Proposed route for the formation of all of the different products of the hydrolysis of allene **2**.

hydrolytic data reported here show that the rates of formation of **1** from **6** (half-lives ($t_{1/2}$) of 32 and 48 h at pH 3.0 and 3.2, respectively) are lower than those from **5** (half-lives of 40 and 65 h, respectively at the same pH values) (**Table 1**). This indicates that at least two separate pathways might be involved in the conversion of **5** to damascenone and that acetylene **6** is not an obligatory intermediate in this conversion (*18*).

Presumably, the delocalized C-3 cation **27** (**Figure 7**) formed by loss of the C-3 hydroxyl in **5** subsequently either loses the C-8 proton to yield the acetylenic alcohol **6**, or undergoes hydration at C-7 followed by tautomerism (with concomitant loss of water) to directly give damascenone (**1**).

The hydrolytic experiments with the model allenic alcohols **7** and **8**, which are 3-deoxy analogues of the polyols **5** and **2**, respectively, indicate that the conversion of simple secondary α-hydroxy allenes to α,β-unsaturated ketones under mild acid conditions is much slower than the reaction of the damascenone precursors **2**, **5**, and **6**. Even when reaction at C-9 took place, as in the formation of the ethyl ether **11** from the alcohol **8**, no transposition of oxygen from C-9 to C-7 was observed. It seems unlikely, therefore, that such transpositions account for damascenone formation in nature.

These observations, together with the results of the earlier hydrolytic experiments (*7, 9, 10*) give a clearer picture of the formation of the various end products from the allenic triol **2**. The formation of all such products is presumably initiated exclusively by acid-catalyzed breakage of the tertiary C-5 carbon−oxygen bond (**Figure 7**) to give cation **25**. Loss of the C-8 proton would lead to formation of the enynediol **4**, while hydration at C-7 would ultimately give 3-hydroxydamascone (**3**). This mechanism for the formation of these two main hydrolysis products is analogous to the two mechanisms proposed above for the formation of damascenone from diol **5**. The formation of diol **5** from triol **2** is relatively minor and results from the loss of the C-4 proton. The hydrolytic studies with the model compounds **7** and **8** indicate that, under mild acid conditions and room temperature, 1,3-transposition of oxygen from secondary allenic alcohols to form α,β-unsaturated ketones does not take place and only occurs with highly conjugated acetylenic precursors such as **6** or tertiary allenic alcohols such as **2** (*8*). Small amounts of the hydrate **14** and ether **15** observed in some hydrolysates appear to be end products rather than intermediates in these transformations as their reactivity at pH 3 is relatively low.

Effects of Glycoconjugation. We have previously compared the rates of hydrolysis of alcohols and their corresponding glycosides (*5*). In the case of geraniol and its *O*-β-D-glucoside, introduction of the sugar unit produced an approximate 10-fold reduction in the rate of linalool formation. Similarly, the rate of formation of damascenone from the enyne diol **4** was some 8 times more rapid than from its C-9 *O*-β-D-glucoside **13**. However, direct comparison of the these rate ratios between geraniol/geranyl glucoside and **4/13** is not strictly appropriate, as in the latter case damascenone is the result of a multistep sequence of transformations, and without direct knowledge of the kinetics of the individual intervening steps, the actual effect of the sugar on the ionization of the C-9 alcohol function is impossible to determine accurately.

The effect of glycosylation on the rate of damascenone formation from the acetylenic alcohol **6** (an approximately 2-fold reduction) was small. Again, as it is not known whether cleavage of the C-9-oxygen bond is rate determining, it is not possible to estimate the effect of glucosylation on this step. The other damascenone precursor studied, **5**, showed a rate reduction of 1.6 upon introduction of glucose at C-3. This ratio was also determined by monitoring the production of damascenone, which again, is the product of several interconversions. In our previous study concerning the hydrolysis of the aglycones **5** only (*10*), it was observed that when diastereomerically pure samples of **5** were hydrolyzed in model wine (pH 3.0) and the reaction was interrupted early (i.e., before one half-life had elapsed), the starting material was accompanied by a second diastereomer of **5**. Chiral GC analysis showed that this new diastereomer had arisen from exclusive epimerization at C-3.

The fact that the proportions of the original diastereomer and the new epimer were equal indicated that complete epimerization had occurred and that epimerization was occurring much more rapidly than conversion into damascenone (**1**). Thus, despite the latter step(s) being rate-determining, the fact that glycosylation at C-3 influenced the rate of formation of damascenone indicates that the true effect of introduction of the sugar on reactivity at this position could be much larger than the observed rate ratio of 1.6 would suggest.

Most polyol precursors to volatile aroma compounds accumulate in plants as glycoconjugates, and while chemical studies on aglycones are useful in understanding precursor-product relationships, it is the behavior of these glycoconjugates that will determine the rate and extent of formation of important odorants such as damascenone. Our studies indicate that the effect of glycosylation on the behavior of the allenic triol in mild aqueous acid might not be important on the time scale of wine making and maturation. The effect of glycosylation at C-3 or C-9 of the triol **2** on product distribution remains undetermined. Conceivably, either steric or inductive effects could influence the degree to which glycosylation of the triol affects the competing steps shown in **Figure 7**. However, the evidence to hand suggests that glycosylation is unlikely to affect product distribution by influencing the relative reactivity of the three carbon−oxygen bonds in **2** as we had previously speculated (*10*).

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Supporting Information Available: ^{1}H NMR, HRMS (ESI), and ^{13}C NMR data for the compounds studied. This material is available free of charge via the Internet at http://pubs.acs.org.

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