JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Precursors to Damascenone: Synthesis and Hydrolysis of Isomeric 3,9-Dihydroxymegastigma-4,6,7-trienes

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A series of four isomeric 3,9-dihydroxymegastigma-4,6,7-trienes, **8**, has been prepared. The (3*S*,6*R*,9*S*) isomer of **8** proved to be identical to an isomer of this compound tentatively identified as an intermediate in the formation of damascenone from an allene triol. Each of the four isomers, when hydrolyzed independently of each other at pH 3.0 and 25 °C, produced product mixtures in which the major product was damascenone (**1**). Contrary to expectation, 3-hydroxydamascone (**5**) was not observed in any of the hydrolyses. Consequently, the mechanism of formation of damascenone proposed earlier requires modification. In each hydrolysis, the product mixtures showed the presence of a second isomer of **8**, produced by epimerization during hydrolysis. Chiral analysis on a Cyclosil B column revealed that this epimerization was occurring at C_3 in each of the hydrolyses.

KEYWORDS: Damascenone; flavor precursors; wine; carotenoid metabolites; epimerization; acid hydrolysis

INTRODUCTION

Since its discovery in the extract of Bulgarian rose (*Rosa damascena*) oil some 30 years ago, damascenone (**1**) has been identified in many different types of plant material and has also been one of the mainstays of the international perfume industry (*1*). It is an extremely potent odorant, with measured aroma detection thresholds of 50 ng L^{-1} in model wine (*2*) and as low as 2 ng/L in water (*3*). Despite its importance to the flavor industry, the biogenesis of naturally occurring damascenone remains unclear. Evidence suggests that damascenone is formed in vivo as the product of degradation of carotenoids such as neoxanthin (**2**, **Figure 1**) (*4*). The allenic triol species **4**, formally derived by reduction of the C_9 carbonyl in grasshopper ketone (**3**), has previously been shown to produce, under mild acid conditions, damascenone (**1**), 3-hydroxydamascone (**5**), and the enyne diol **6**, with **1** being formed only in very minor amounts (*6*). The enyne **6** was also found to produce both **1** and **5**, albeit at a greatly reduced rate (*7*); hydrolytic data indicated that **6** produces, in one year, approximately the same quantity of damascenone as the triol **4** produces in 1 day. Somewhat surprisingly, perhaps, **5** has been found to be extremely resistant toward dehydration, even under strongly acidic conditions (*8*), indicating that it is a terminal product in this reaction sequence, rather than an intermediate in the eventual formation of **1**. Glycoconjugated forms of both **4** (*9*) and **6** (*10*, *11*) have been isolated from various plants, and both have been shown to lead to the production of damascenone.

Earlier work in our laboratories has shown the effect of glycoconjugation on the reactivity of activated hydroxyl functions. For example, the enyne diol **6** produced both **1** and **5** in a ratio of 1:17 when heated at 50 °C in a pH 3.0 buffer (*12*). In contrast, when the corresponding C_9 β-D-glucopyranoside of **6** was heated under identical conditions, the proportion of damascenone formed increased to 1:11. Furthermore, the rate of hydrolysis of the glucoside was approximately $^1/_{10}$ that of the aglycon (*7*). These results have been interpreted as showing that the acid-catalyzed ionization of an activated hydroxyl function is suppressed when that position is glycoconjugated. Consequently, other processes might become competitive, in this case, loss of the C_3 hydroxyl leading to formation of **1**. Thus, the site of glycosylation might strongly influence the formation of **1** from precursors such as **4**.

These earlier studies also provided tentative evidence for the intermediacy of two new compounds, the dienyne **7** and the allenic diol **8**, in the formation of **1** from **4** (*6*). On the basis of this evidence, a mechanism for the formation of the hydrolysis products from **4** was proposed as shown in **Figure 2**. Loss of the C_5 alcohol concomitant with rearrangement produces **6**, whereas direct elimination of the C_5 hydroxyl would produce the allenic diol **8**. From this point it could be anticipated that reaction might proceed through *either* the C_3 *or* C_9 hydroxyls. Loss of the former would be expected to produce damascenone, whereas loss of the latter would be expected to furnish **5**. The

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10.1021/jf050327p CCC: $30.25 © 2005 American Chemical Society
Published on Web 04/30/2005

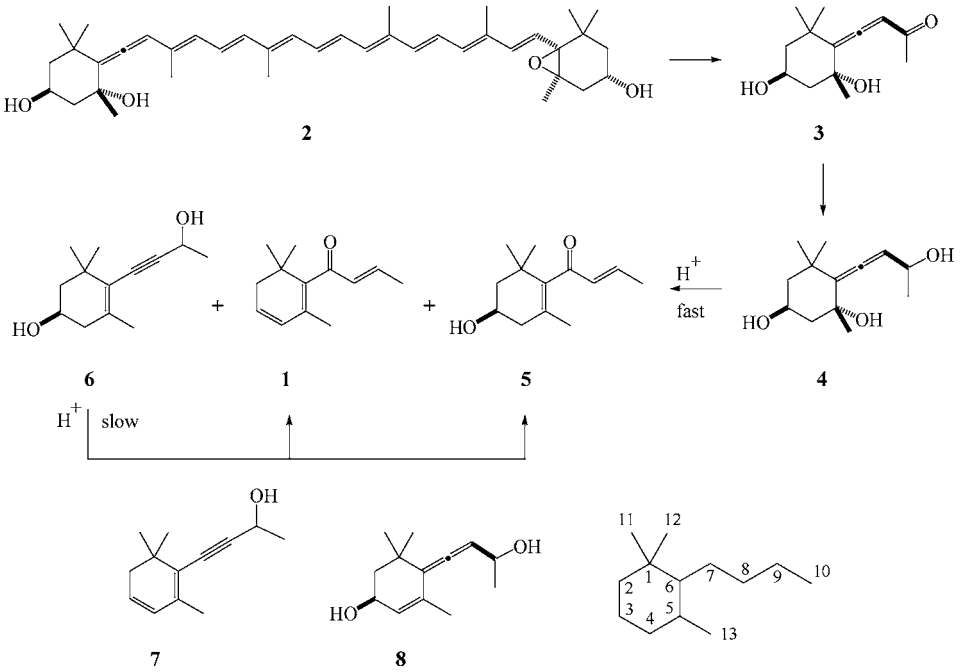

Figure 1. Proposed pathway for the formation of **1** from neoxanthin (**2**), including the structures of two tentatively identified intermediates, **7** and **8**, and the generic numbering scheme employed for megastigmane derivatives (*5*).

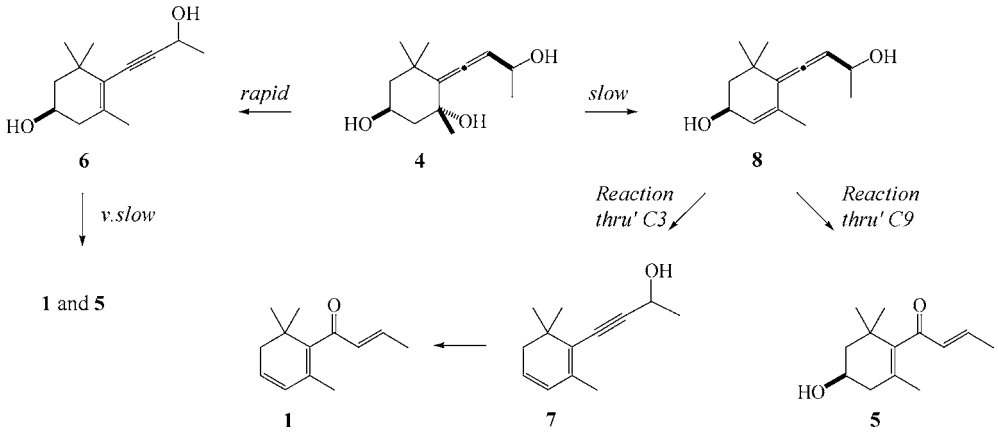

Figure 2. Proposed mechanism for hydrolysis of triol **4**.

ratio of products formed would then be dependent upon the relative reactivity of the two alcohols toward acid-catalyzed ionization.

Recently we reported the synthesis of an authentic sample of **7** and confirmed its identity as one of the intermediates observed in the hydrolysis of **4** (*4*). In addition, the product of its hydrolysis proved to be almost exclusively (>90%) damascenone **1**, confirming **7** as the immediate precursor to naturally formed damascenone. The remainder (<10%) comprised two compounds, which had previously been observed in the hydrolysis of damascenone precursors and which were assigned as the C_9 hydrate of damascenone **13** and the C_9 ethanol adduct **14**.

Comparisons between the allenic triol **4** and the diol **8** are revealing: in the former, the C_3 position is not activated, unlike the other two oxygenated positions, and the major hydrolysis products of **4** (i.e., **5** and **6**) both retain the C_3 hydroxyl. In the diol **8**, on the other hand, both C_3 and C_9 are activated, and one would expect formation of damascenone from **8** to be competitive with the formation of **5**.

Thus, our aims in conducting this study were twofold: first, synthesis and characterization of the allenic diol **8** and confirmation of its identity from the earlier study and, second, a thorough investigation into the hydrolytic behavior of this compound. It was anticipated that these authentic allene diols would produce mixtures consisting principally of damascenone and its hydroxylated derivative **5** and that the ratio of these two products would be determined by the relative reactivity toward ionization of the hydroxyl functional groups located at either C_3 or C_9.

MATERIALS AND METHODS

Materials. Chemicals were purchased from Sigma-Aldrich. (*S*)-Phorenol (ee > 96%) was provided by Dr. K. Puntener, Hoffmann-La Roche International. All solvents used were of pesticide grade from OmniSolv (Darmstadt, Germany). All organic solvent solutions were dried over anhydrous sodium sulfate before being filtered. pH measurements were made with an EcoScan pH 5/6 meter (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. 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. (*17*). Buffer solutions were prepared by saturating a 10% ethanol solution with potassium hydrogen tartrate and adding 10% tartaric acid solution until the required value was reached.

Methods. *(S)-4-tert-Butyldimethylsilyloxy-2,6,6-trimethylcyclohex-2-enone [(S)-**10**].* To a solution of (*S*)-phorenol (**9**) (1.0 g, 6.40 mmol) in pyridine (10 mL) was added *tert*-butyldimethylsilyl chloride (1.47 g, 9.60 mmol). The mixture was stirred at room temperature overnight, quenched with water, and extracted with ethyl acetate. The organic layer was washed with brine and dried (Na_2SO_4) before being concentrated in vacuo to yield material that was used without further purification (1.60 g, 92%): $[\alpha]_D$ −49.0 (*c* 0.40, $CHCl_3$) [lit. (*18*) −57 (*c* 0.44, $CHCl_3$]; δ_H 6.49 (1H, m, H_3), 4.56 (1H, m, H_4), 1.99 (1H, ddd, J = 13.0, 5.5, and 1.9 Hz, H_{5a}), 1.87 (1H, dd, J = 13.0 and 9.6 Hz, H_{5b}), 1.78−1.76 (3H, m, H_9), 1.14, 1.11 (6H, 2s, $H_{7,8}$), 0.92 (9H, s, *t*-Bu), 0.13, 0.11 (6H, 2s, SiMe).

*(3S,9S)-Dihydroxymegastigma-4,6,7-triene [(3S,9S)-**8**].* These reactions were first optimized using both **10** and but-3-yn-2-ol in racemic form. The products of these reactions were necessarily mixtures of diastereomers, but gave entirely satisfactory spectroscopic and microanalytical data: Anal. (**11**) Calcd for $C_{19}H_{34}O_3Si$: C, 67.4; H, 10.1. Found: C, 67.2; H, 10.0. Anal. (**12**) Calcd for $C_{19}H_{34}O_2Si$: C, 70.7; H, 10.6. Found: C, 70.7; H, 10.7.

(*S*)-But-3-yn-2-ol (0.31 g, 4.50 mmol) in ether (200 mL) was treated with *n*-BuLi (2.5 M, 3.5 mL, 8.6 mmol) at 0 °C and stirred at room temperature for 2 h. (*S*)-**10** (0.40 g, 1.6 mmol) was then added, and the mixture was stirred at room temperature for 48 h before being quenched with saturated NH_4Cl solution. The residue was extracted with diethyl ether, washed with brine, dried (Na_2SO_4), and concentrated in vacuo. The crude material was purified by column chromatography (30% ethyl acetate/hexanes) to yield (3*S*,9*S*)-**11** as a colorless oil (0.35 g, 69%). NMR analysis revealed the product to be a mixture of two diastereomers.

(3*S*,9*S*)-**11** (0.25 g, 0.73 mmol) in ether (10 mL) was treated with $LiAlH_4$ (0.14 g, 3.7 mmol) and the mixture stirred at reflux for 4 h. The reaction was quenched by the addition of a solution of saturated sodium sulfate, and the product was extracted with ethyl acetate. The organic extracts were then washed with 10% NaOH solution and brine, before being dried (Na_2SO_4) and concentrated in vacuo. The residue was purified by column chromatography (10% ethyl acetate/hexane) to yield (3*S*,9*S*)-**12** as a pair of diastereomers (85 mg, 36%). The product was treated with *tert*-butylammonium fluoride (*t*-BAF) (0.53 mL of 1.0 M solution in THF, 0.53 mmol) in dichloromethane (10 mL) overnight at room temperature, before being quenched with saturated $NaHCO_3$ solution and extracted with ethyl acetate. The extract was washed with brine, dried, and concentrated in vacuo to yield 80 mg of crude material. Purification by chromatography (40% ethyl acetate, hexane) yielded pure *SS*-**1** isomer (12 mg, 23%), followed by a mixed fraction (27 mg, 50%), followed by a fraction containing pure *SS*-**2** isomer (8 mg, 14%).

SS-1 isomer, (3*S*,6*S*,9*S*)-**8**: $[\alpha]_D$ −22.6 (*c* 0.02, $CHCl_3$); δ_H 5.66 (1H, br d, J = 5.3 Hz, H_8), 5.60 (1H, m, H_4), 4.42−4.28 (2H, m, $H_{3,9}$), 1.91 (1H, ddd, J = 12.5, 5.8, and 1.1 Hz, H_{2a}), 1.74 (3H, app t, $J \sim$ 1.6 Hz, H_{13}), 1.60 (2H, br s, OH), 1.43 (1H, dd, J = 12.5 and 9.8 Hz, H_{2b}), 1.31 (3H, d, J = 6.3 Hz, H_{10}), 1.12, 1.07 (6H, 2s, $H_{11,12}$); δ_C 199.9, 130.3, 128.0, 116.3, 101.6, 66.2, 65.9, 45.9, 33.8, 29.7, 28.3, 23.5, 20.9; MS, *m*/*z* (%) 208 (4), 193 (5), 190 (22), 175 (43), 157 (16), 149 (35), 146 (24), 131 (100), 121 (26), 115 (35), 109 (32), 105 (30), 91 (53), 77 (26), 69 (24).

SS-2 isomer, (3*S*,6*R*,9*S*)-**8**: $[\alpha]_D$ +45.0 (*c* 0.02, $CHCl_3$); δ_H 5.61 (1H, m, H_4), 5.58 (1H, br d, J = 6.0 Hz, H_8), 4.42−4.28 (2H, m, $H_{3,9}$), 1.92 (1H, ddd, J = 12.3, 5.8, and 1.1 Hz, H_{2a}), 1.75 (3H, app t, $J \sim$ 1.6 Hz, H_{13}), 1.60 (2H, br s, OH), 1.45 (1H, dd, J = 12.3 and 9.6 Hz, H_{2b}), 1.31 (3H, d, J = 6.3 Hz, H_{10}), 1.12, 1.05 (6H, 2s, $H_{11,12}$); δ_C 200.1, 130.1, 127.9, 116.1, 101.4, 66.4, 65.9, 46.1, 33.9, 30.1, 27.7, 23.5, 20.8; MS, *m*/*z* (%) 208 (3), 193 (4), 190 (11), 175 (20), 157 (12), 149 (20), 146 (28), 131 (100), 121 (16), 115 (29), 109 (28), 105 (19), 91 (44), 77 (21), 69 (16).

*(3S,9R)-Dihydroxymegastigma-4,6,7-triene [(3S,9R)-**8**].* The above reaction sequence was repeated using (*R*)-but-3-yn-2-ol (0.31 g, 4.5 mmol) and (*S*)-**10** (0.40 g, 1.6 mmol). Identical workup gave (3*S*,9*R*)-**11** as a colorless oil (0.35 g, 69%), again as a pair of diastereomers.

(3*S*,9*R*)-**11** (0.25 g, 0.73 mmol) was treated with $LiAlH_4$ (0.14 g, 3.7 mmol) in ether (10 mL) as described above. Workup gave, after chromatography (3*S*,9*R*)-**12** (64 mg, 23%) as a colorless oil. Deprotection with *t*-BAF (0.40 mL of 1.0 M solution in THF, 0.40 mmol) provided 74 mg of crude material, which was chromatographed as before to give, in order of elution, pure **SR-1** isomer (16 mg, 40%), a mixed fraction (19 mg, 47%), and pure **SR-2** isomer (4 mg, 10%).

SR-1 isomer, (3*S*,6*S*,9*R*)-**8**: $[\alpha]_D$ +12.8 (*c* 0.25, $CHCl_3$); δ_H 5.66 (1H, br d, J = 5.4 Hz, H_8), 5.60 (1H, m, H_4), 4.41−4.29 (2H, m, $H_{3,9}$), 1.91 (1H, ddd, J = 12.5, 5.8, and 1.1 Hz, H_{2a}), 1.75 (3H, app t, $J \sim$ 1.6 Hz, H_{13}), 1.62 (2H, br s, OH), 1.42 (1H, dd, J = 12.5 and 9.6 Hz, H_{2b}), 1.31 (3H, d, J = 6.3 Hz, H_{10}), 1.11, 1.07 (6H, 2s, $H_{11,12}$); δ_C 199.7, 130.1, 128.0, 116.0, 101.5, 66.2, 65.8, 45.8, 33.7, 29.6, 28.2, 23.5, 20.9; MS, *m*/*z* (%) 208 (5), 193 (10), 190 (23), 175 (45), 157 (15), 149 (29), 146 (30), 131 (100), 121 (30), 115 (39), 109 (26), 105 (27), 91 (45), 77 (28), 69 (22).

SR-2 isomer, (3*S*,6*R*,9*R*)-**8**: $[\alpha]_D$ +35.2 (*c* 0.06, $CHCl_3$); δ_H 5.62−5.55 (2H, m, $H_{4,8}$), 4.41−4.30 (2H, m, $H_{3,9}$), 1.92 (1H, ddd, J = 12.3, 5.7, and 1.1 Hz, H_{2a}), 1.74 (3H, dd, J = 1.6 and 1.3 Hz, H_{13}), 1.61 (2H, br s, OH), 1.45 (1H, dd, J = 12.3 and 9.6 Hz, H_{2b}), 1.31 (3H, d, J = 6.4 Hz, H_{10}), 1.13, 1.05 (6H, 2s, $H_{11,12}$); δ_C 199.9, 130.2, 128.0, 116.2, 101.5, 66.4, 65.9, 46.1, 33.9, 30.0, 27.6, 23.5, 20.8; MS, *m*/*z* (%) 208 (4), 193 (3), 190 (15), 175 (30), 157 (9), 149 (25), 146 (34), 131 (100), 121 (20), 115 (37), 109 (22), 105 (23), 91 (48), 77 (27), 69 (20).

General Hydrolysis Procedure. Solutions of **8** (1 mg/L) in buffered 10% aqueous ethanol at pH 3.0 were sealed in ampules and heated at 25 °C in a water bath for 24 h. After this time, ampules were removed and opened, and the contents were extracted with ether and subjected to GC-MS analysis, on either an achiral DB-1701 column or a chiral Cyclosil B column, using the conditions reported by Wilkinson et al. (*19*). Several ampules of each were retained in the water bath for 6 months, after which time they were opened and analyzed.

*Hydrolysis of **8** (Details Given for the **SS-2** Isomer; Others Conducted in an Identical Manner).* A solution of (3*S*,6*R*,9*S*)-**8** (1 mg/L, 10 mL aliquots) was prepared, heated, and extracted as described above. The product mixture was shown to contain **1**, **7**, hydrate **13**, ethanol adduct **14**, unreacted **SS-2** isomer, a second isomer of **8** that proved to be indistinguishable from the synthetic **SR-1** isomer, and two isomers of a compound for which mass spectra were in accord with the assigned structure **15**; isomer 1, *m*/*z* (%) 236 (24), 221 (100), 203 (10), 191 (21), 177 (50), 149 (26), 147 (31), 146 (30), 137 (94), 131 (77), 121 (35), 109 (63), 105 (41), 91 (45), 77 (28), 69 (21); isomer 2, *m*/*z* (%) 236 (23), 221 (100), 203 (10), 191 (19), 177 (49), 149 (26), 147 (28), 146 (23), 137 (91), 131 (57), 121 (34), 109 (60), 105 (38), 91 (41), 77 (25), 69 (21).

Compounds **1**,[6] **7**,[4] **13**,[14] and **14**[14] were identified by comparison of retention times and mass spectra with those of authentic samples.

RESULTS AND DISCUSSION

Synthesis of Allenes 8. The synthesis of the allenic diols (**Figure 3**) was based on a strategy that involved as little stereochemical manipulation as was practicable. By using a starting reagent with fixed (*S*) stereochemistry at C_3, the stereochemistry at C_9 can be introduced by selective use of optically active 3-butyn-2-ol. This would allow the preparation of four of the possible eight diastereomers of **8**, necessary for a proper evaluation of their hydrolytic behavior.

The hydroxyl in (*S*)-phorenol (**9**) was protected as its TBS ether and then treated with the dilithio derivative of either (*S*)-3-butyn-2-ol or the enantiomeric (*R*) analogue (*13*). Each reaction produced a pair of isomers of **11**, which differed in their stereochemistry at C_6. The individual diastereomers were not isolated for either reaction, but rather each was treated with LAH to produce a pair of isomers epimeric at the allenic position (*20*). Neither the pair of isomers of **12** produced from the (*S*)-

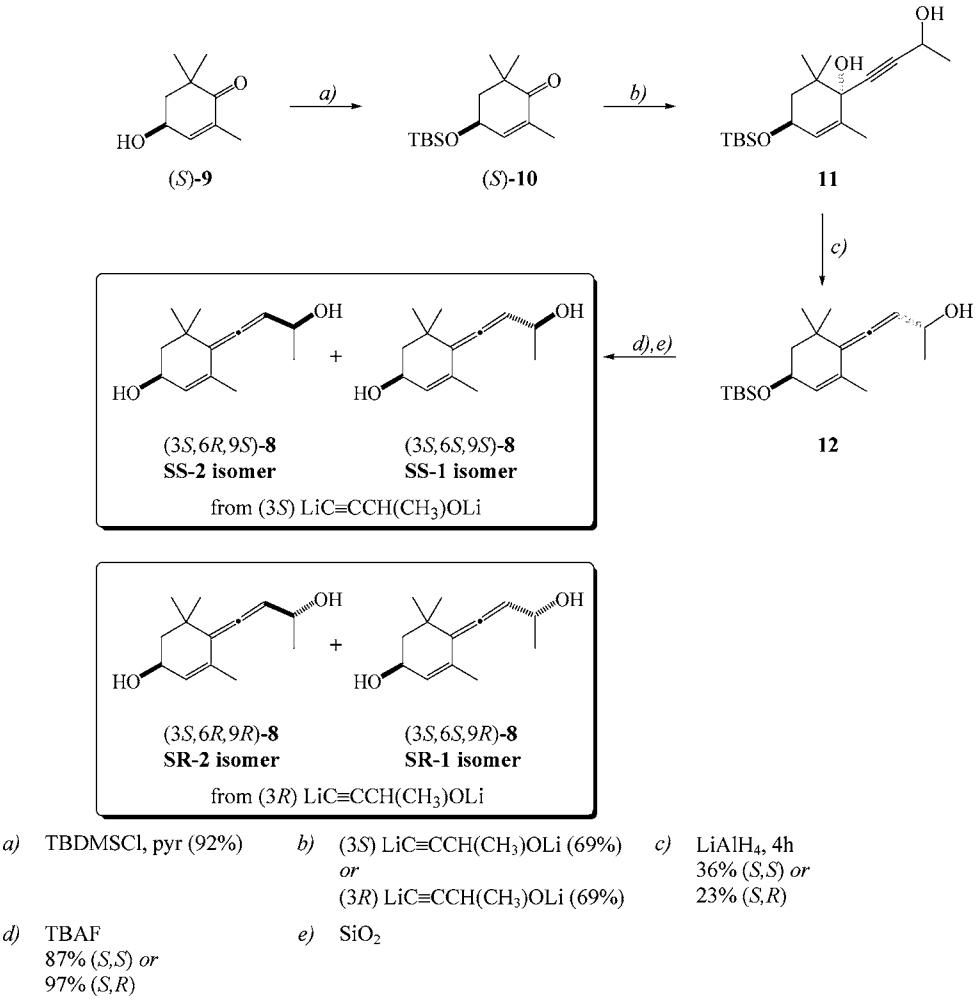

Figure 3. Synthesis of target allenes from (*S*)-phorenol (**9**).

3-butyn-2-ol series of reactions nor the pair from the corresponding (*R*)-3-butyn-2-ol series could be adequately separated at this stage. However, after removal of the silyl protecting group, each series of compounds was separable by column chromatography. The two isomers of **8** produced from the (*S*)-3-butyn-2-ol series of reactions were labeled **SS-1** and **SS-2**, with the numerical descriptor referring to the order of elution during column chromatography on silica. The assignment of C_6 stereochemistry to these two isomers is discussed in the next section. Similarly, the two isomers of **8** produced from the (*R*)-3-butyn-2-ol series of reactions were labeled **SR-1** and **SR-2**. All four synthetic isomers were distinguishable from one another by GC-MS, with the order of elution on a DB-1701 column being **SR-2**, **SS-2**, **SS-1**, and **SR-1**.

Hydrolysis of Allenes 8. The first issue to be resolved concerned the identity of the compound tentatively identified as **8**, from the original hydrolysate of **4**. The original triol **4** was synthesized by Skouroumounis et al. (*15*) from diastereomerically pure [3′*R**,1*R**,4*S**,6*S**]-1-(3′-hydroxybut-1′-ynyl)-2,2,6-trimethylcyclohexane-1,4-diol, which was fractionally crystallized from a mixture and for which relative stereochemistry was established by X-ray crystallography (*16*). This diastereomer was used for the conversion to **4**, with the relative stereochemistry at C_3 and C_9 remaining unaltered.

Given that the original hydrolysis experiments were conducted on material that was diastereomerically homogeneous and that the relative stereochemistry at all positions was known, then a match with one of the authentic (3*S*,9*S*) allene isomers **8** would be expected. Accordingly, when compared by GC-MS analysis (retention time, mass spectrum, and co-injection), the second of these isomers (**SS-2**) provided an exact match with the compound tentatively identified as **8** from the hydrolysate of **4**, and we are thus able to assign stereochemistry to the (3*S*,9*S*) allenes: **SS-1** as (3*S*,6*S*,9*S*)-**8** and **SS-2** as (3*S*,6*R*,9*S*)-**8**.

Each of the four allene diols **8** was subjected to acid hydrolysis, and the products were examined after 24 h and 6 months of reaction time. Some general features were observed in the products; in the case of the long-term hydrolysis, the products were damascenone (**1**) and the addition products **13** and **14**, in a ratio similar to that observed previously in the hydrolysis of **7** (*4*). However, when the short-term hydrolyses were examined, the major products (at the same oxidation level as damascenone) were damascenone **1**, intermediate **7**, unreacted **8** (plus epimer), two compounds tentatively identified as epimers of **15**, and minor amounts of damascenone addition products **13** and **14** (**Figure 4**). Minor amounts (<10%) of several oxidation products were also observed. Noteworthy in each case was the complete absence of 3-hydroxydamascone (**5**), even in trace amounts. This result demanded reappraisal of the original mechanism proposed by Skouroumounis and Sefton for the formation of **1**, **5**, and **6** (*7*).

Each allene diol **8** showed, after 24 h, considerable reaction progress (~40% consumption of **8**), with unconsumed allene diol now accompanied by an equal amount of a compound that was clearly epimeric, on the basis of mass spectrometric analysis. Each isomer of **8** gave only a single new epimer of **8**; in the case of the **SS-2** isomer, this epimer proved to be indistinguishable from the **SR-1** isomer by achiral GC. This

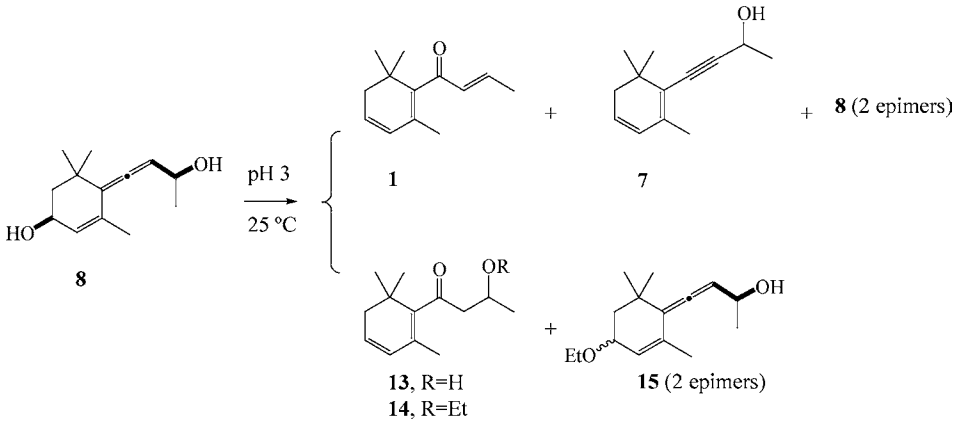

Figure 4. Products of the interrupted hydrolysis of allenes **8**.

Table 1. Absolute Stereochemistries Expected for Epimerization at either C_9 or C_3 during Hydrolysis of **8** and Retention Times Observed on a Chiral Stationary Phase

starting isomer	after epimerization[b]	after epimerization[a] at C_9	after epimerization[a] at C_3	GC retention times of isomers of **8**[c]
SS-1 (= 3*S*,6*S*,9*S*)	**SS-1** + **SR-2**[d]	(3*S*,6*S*,9*S*) + (3*S*,6*S*,9*R*)	(3*S*,6*S*,9*S*) + (3*R*,6*S*,9*S*)	each 18.09
SS-2 (= 3*S*,6*R*,9*S*)	**SS-2** + **SR-1**[d]	(3*S*,6*R*,9*S*) + (3*S*,6*R*,9*R*)	(3*S*,6*R*,9*S*) + (3*R*,6*R*,9*S*)	18.10 and 18.17
SR-1	**SR-1** + **SS-2**[d]	(3*S*,6*R*,9*R*) + (3*S*,6*R*,9*S*)	(3*S*,6*S*,9*R*) + (3*R*,6*S*,9*R*)	each 18.13
SR-2	**SR-2** + **SS-1**[d]	(3*S*,6*S*,9*R*) + (3*S*,6*S*,9*S*)	(3*S*,6*R*,9*R*) + (3*R*,6*R*,9*R*)	18.07 and 18.15

[a] Stereochemistries expected after epimerization at either C_3 or C_9. [b] Isomers of **8** present after hydrolysis interrupted at 24 h, as determined on an achiral (DB-1701) GC column. [c] Retention times (minutes) of isomers of **8** present after hydrolysis interrupted at 24 h, as determined on a chiral (Cyclosil B) GC column. [d] Or its enantiomer.

pairing of the **SS-2** isomer with the **SR-1** isomer was observed in the converse reaction also. Similarly, the **SS-1** isomer gave an epimer indistinguishable from the **SR-2** isomer, and vice versa. **Table 1** lists the epimeric pairs obtained after hydrolysis of each of the four allene diastereomers.

Whether this epimerization is taking place at C_3 or C_9 is impossible to determine from these data alone. For instance, if epimerization is occurring at C_9 of the **SS-2** isomer, then the new epimer will have (3*S*,6*R*,9*R*) stereochemistry. Conversely, if this epimerization is occurring at C_3, the new epimer will have (3*R*,6*R*,9*S*) stereochemistry and will be the enantiomer of the synthetic isomer having (3*S*,6*S*,9*R*) stereochemistry. Thus, the ultimate assignment of stereochemistry to the **SR-1** and **SR-2** isomers is reliant upon our being able to distinguish between C_3 and C_9 epimerization. **Table 1** also shows the absolute stereochemistry of the products that would be formed from each of the two processes (i.e., C_3 or C_9 epimerization). If epimerization takes place at C_9, then the pair of products from the **SS-2** isomer of **8** would have the *same* absolute stereochemistry as the pair formed from the **SR-1** isomer, whereas these pairs of products would have *differing* absolute stereochemistries if epimerization were at C_3. Similarly, the **SS-1** and **SR-2** isomers would give pairs of products with the same absolute stereochemistry only for C_9 epimerization.

Whereas the four diastereomers of **8** cannot be distinguished from their corresponding enantiomers by achiral GC, chiral GC offers scope for resolving such isomers. The retention times for gas chromatography on a chiral Cyclosil B column of the isomers of **8** formed in the hydrolysates are also shown in **Table 1** and demonstrate that epimerization took place at C_3. That these results were not due to minor variation in chromatographic behavior of the isomers was demonstrated by rerunning all of the samples in a different order, on a separate occasion, with identical outcomes. We are thus able to confirm the absolute stereochemistries for the two (3*S*,9*R*) allenes: **SR-1** as (3*S*,6*S*,9*R*)-**8** and **SR-2** as (3*S*,6*R*,9*R*)-**8**.

Compounds **15** were assigned as being ethyl ethers of **8**, primarily on the basis of their mass spectra. The masses (*m*/*z* 236, M^+) and fragmentation patterns are entirely consistent with this assignment. Additional evidence comes from the epimerization of **8** itself; protonation and ionization at C_3 of **8** followed by capture by either water or ethanol provide an entirely reasonable pathway to these compounds.

The fact that epimerization takes place only at C_3 under the conditions used in this study (conditions chosen for their similarity to normal wine maturation) has implications for the mechanism of formation of damascenone. The original proposal (**Figure 2**) was consistent with the available data but needs to be revised in light of the new findings. The suggestion that both **5** and **7** are formed from a common intermediate, **8**, is contradicted by the finding that the hydrolysate produced from authentic **8** is completely devoid of **5**. This compound, the major product in the hydrolysis of allenic triol **4**, appears to be formed directly from **4**, via a process as yet undetermined.

ACKNOWLEDGMENT

We are very grateful to Dr. K. Puntener, Hoffmann-La Roche International, for supplying a sample of authentic (*S*)-phorenol (ee > 96%). We thank Profs. I. S. Pretorius and P. B. Høj, as well as Dr. E. J. Waters, for support and feedback.

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Received for review February 14, 2005. Revised manuscript received April 5, 2005. Accepted April 7, 2005. This project is supported by Australia's grapegrowers and winemakers through their investment body, the Grape and Wine Research and Development Corporation, with matching funds from the Australian government, and by the Commonwealth Cooperative Research Centres Program. The work was conducted by The Australian Wine Research Institute and Flinders University as part of the research program of the Cooperative Research Centre for Viticulture.

JF050327P