Model Studies of the Biosynthesis of Non-Head-to-Tail Terpenes. Stereochemistry of Ionization for *N*-Methyl-4-[(1*S*,1′*R*,3′*R*)-[1-^{2}H]chrysanthemyloxy]-pyridinium Iodide[1]

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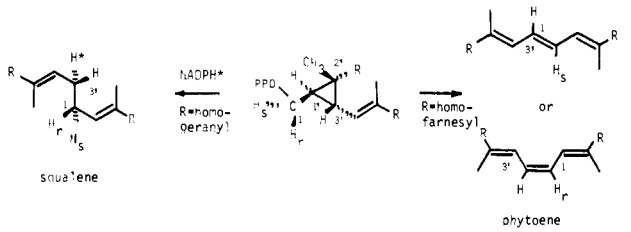
Abstract: Hydrolysis of *N*-methyl-4-[(1*S*,1′*R*,3′*R*)-[1-^{2}H]chrysanthemyloxy]pyridinium iodide (**1-OPyI-^{2}H$_1$**) is stereoselective with 86% of the ionization occurring from a conformation where the carbon–oxygen bond is antiperiplanar to the C(1′)–C(3′) cyclopropane bond. In contrast, ionization from the antiperiplanar conformation in *N*-methyl-4-[(1*S*,1′*R*,3′*R*)-[1-^{2}H]-dihydrochrysanthemyloxy]pyridinium iodide (**2-OPyI-^{2}H$_1$**) only constitutes 51% of the total. The effect of the double bond on the stereochemistry at C(1) is discussed in regard to the chemistry of the chrysanthemyl cation and the stereospecific enzyme-catalyzed head-to-head rearrangements found in the sterol and carotenoid biosynthetic pathways.

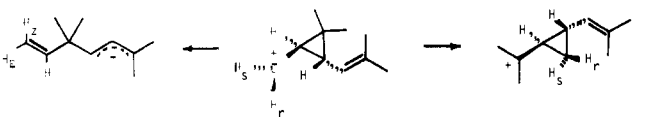
The stereospecific head-to-head condensation of two molecules of farnesyl pyrophosphate in the sterol biosynthetic pathway[3] and geranylgeranyl pyrophosphate in the carotenoid biosynthetic pathway[4] proceeds through cyclopropylcarbinyl intermediates presqualene pyrophosphate (R = $C_{11}H_{19}$) and prephytoene pyrophosphate (R = $C_{16}H_{27}$), respectively. The



cyclopropylcarbinyl to head-to-head rearrangement requires cleavage of the C(1′)–C(3′) and C(2′)–C(3′) cyclopropane bonds and formation of a bond between C(1) and C(3′). During the rearrangement which lead to squalene, C(1) and C(3′) are inverted, while (*E*)- and (*Z*)-phytoene retain the *pro-S* and *pro-R* hydrogens at C(1), respectively. We[5] and others[6] have studied the individual steps of a cationic head-to-head rearrangement using C_{10} models. The regiochemical implications of this work have been discussed in terms of possible mechanisms for catalysis by an enzyme.[5,6]

The stereochemistries that are observed during biosynthesis of squalene and phytoene can also be related to the proposed mechanisms in a logical manner.[5] We were interested in determining to what extent the stereochemical preferences exhibited by the vinyl-substituted cyclopropylcarbinyl substrates in the absence of an enzyme were expressed in the biological transformations. The rearrangements that take the chrysanthemyl system to 2,7-dimethylocta-2,6-dien-4-ol were chosen as a model for the conversion of presqualene pyrophosphate to squalene.[5] Work concerning the stereochemistry at C(3′) has been reported.[5c]

In turning our attention to C(1), we find that the cyclopropylcarbinyl to head-to-head rearrangement involves two changes in bonding at that center, cleavage of the C(1)–oxygen bond during ionization, and formation of the C(1)–C(3′) bond during rearrangement. Previous model studies indicate that the chrysanthemyl system rearranges to the isomeric cations shown below.[5a,d] Interchange of the C(1) methylene protons cannot occur after ionization and before rearrangement because of the high barrier to rotation about the C(1)–C(1′) bond in primary cyclopropylcarbinyl cations.[7] Thus, the stereochemistry of ionization is reflected in the relative orientation



of the methylene protons of the terminal double bond of the allylic cation, while the stereochemistry at C(1) in the rearranged tertiary cyclopropylcarbinyl cation is a composite of the stereochemistries of the two individual steps. By determining the stereochemistries for products derived from the rearranged cations, it should be possible to deduce the stereochemistry of rearrangement.

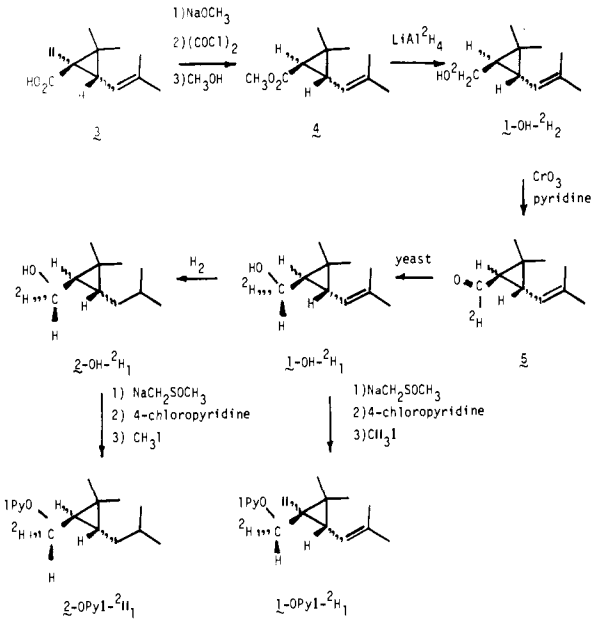
We originally suggested that a conformation in which the C(1)–oxygen bond was antiperiplanar to the C(1′)–C(3′) cyclopropane bond would facilitate ionization by using the vinyl moiety at C(3′) for delocalization of charge.[3a] Subsequent inversion of C(1) is consistent with stereochemical studies of the cyclopropylcarbinyl–cyclopropylcarbinyl rearrangement in alkyl-substituted cations.[3a,5b,c,d] However, recently Wilcox and co-workers[8] reported that cyclopropane rings transmit position charge to attached substituents very poorly, and we found that the double bond at C(3′) only provides a threefold rate enhancement in the chrysanthemyl system.[5a] An additional complication results from the observation that the stabilizing effect of substituents attached to a remote cyclopropane carbon as evidenced by an increased rate of solvolysis cannot be related to the stereochemistry of the ionizing center in a simple way.[9] Thus, it is necessary to determine the stereochemistry at C(1) during ionization by a direct method.[10] In this and the following paper, we provide data which permits us to dissect the overall stereochemistry for rearrangement into its two components.

Results and Discussion

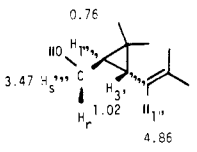
Synthesis and Hydrolysis of *N*-Methyl-4-[(1*S*,1′*R*,3′*R*)-[1-^{2}H$_1$]chrysanthemyloxy]pyridinium Iodide and *N*-Methyl-4-[(1*S*,1′*R*,3′*R*)-[1-^{2}H$_1$]dihydrochrysanthemyloxy]pyridinium Iodide. The compounds used in this study were prepared by the reactions outlined in Scheme I, starting with (1*R*,3*R*)-chrysanthemic acid which was 98% optically pure. The alcohol obtained by reducing methyl ester **4** with lithium aluminum deuteride gave an NMR spectrum that was devoid of absorption in the region of 3.3 ppm, even at high spectrum amplitudes. Care was taken to ensure that the chromium trioxide–pyridine oxidation went to completion in order to avoid any contamination of **1-OH-^{2}H$_1$** by the precursor dideuterio alcohol.

Aldehyde **5** was reduced to (1*S*,1′*R*,3′*R*)-chrysanthemol

Scheme I. Synthesis of 1-OPyl-$^2H_1$ and 2-OPyl-$^2H_1$



in an aqueous suspension of actively fermenting baker's yeast. The NMR spectrum of 1-OH-$^2H_1$ shows that the reduction gave mostly a single stereoisomer at C(1). The protons at C(1)



$\lvert J_{H_s,H_\gamma}\rvert$	= 11.4 Hz
$\lvert J_{H_s,H(1')}\rvert$	= 6.2 Hz
$\lvert J_{H_\gamma,H(1')}\rvert$	= 8.3 Hz
$\lvert J_{H(1'),H(3')}\rvert$	= 5.5 Hz
$\lvert J_{H(1'),H(1'')}\rvert$	= 7.5 Hz

of chrysanthemol are intrinsically nonequivalent with a chemical shift separation of 0.25 ppm in carbon tetrachloride.[11] In 1-OH-$^2H_1$ the eight-line pattern for the hydroxymethyl protons is reduced to a doublet of triplets at δ 3.22 ($J_{^1H,^2H}$ = 1.61 Hz) which is further simplified to a doublet upon irradiation at the deuterium resonance position (Figure 1). These peaks are assigned to the proton at C(1) (H_r) in (1*S*,1′*R*,3′*R*)-[1-^{2}H]chrysanthemol. Yeast alcohol dehydrogenase is known to reduce deuterated aldehydes to the corresponding *S*-primary alcohols.[12] Our assignment was recently confirmed by Popjak and co-workers[13] in a series of experiments whereby (1*S*,1′*R*,2′*R*,3′*R*)-presqualene alcohol was prepared from (1*S*)-farnesol using reactions that did not involve changes in bonding to the carbon atom that becomes C(1) in the cyclopropylcarbinyl alcohol.

A weak doublet at δ 3.47 (J = 6.2 Hz) was expected, since aldehyde **5** contained 2% of the 1*S*,3*S* enantiomer. However, 1-OH-$^2H_1$ and the dihydro alcohol 2-OH-$^2H_1$ obtained from 1-OH-$^2H_1$ by catalytic hydrogenation gave a more intense peak than expected (see Table I). The amount of "mislabel" as determined from the less intense signal could arise from one or both of two situations. If reduction of the deuterio aldehyde was stereoselective, but not stereospecific, all four of the diastereomers listed in Table I would be formed. Otherwise, a stereospecific reduction of **5** with a competing side reaction that selectively depletes the 1*R*,3*R* enantiomer would yield the observed results.[14] Although we cannot distinguish between the two possibilities at the present time, our experiments with 1-OH-$^2H_1$ and 2-OH-$^2H_1$ are not compromised, since it is only necessary to know the relative configuration of C(1) with respect to C(1′) and C(3′).

Hydrolyses of the *N*-methylpyridinium derivatives of 1-OH-$^2H_1$ and 2-OH-$^2H_1$ were carried out at 25 °C in aqueous solutions buffered by sodium bicarbonate. 1-OPyl-$^2H_1$ gave two major products, yomogi alcohol (6-OH-$^2H_1$) and artemisia

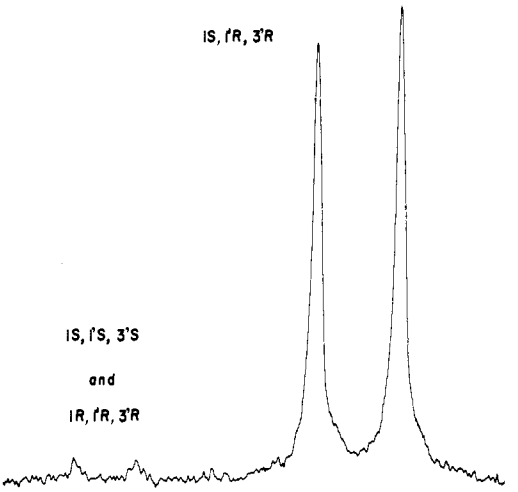
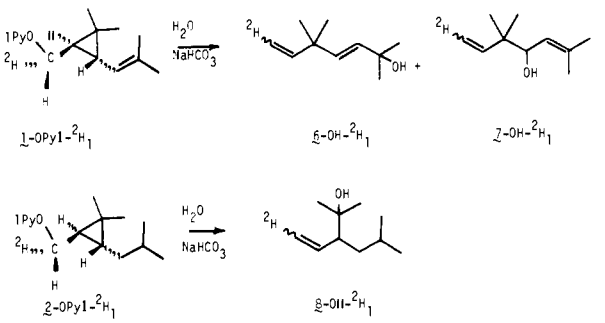


Figure 1. NMR spectrum of the region between 3 and 4 ppm for 1-OH-$^2H_1$.

Table I. Distribution of ^2H in 1-OH-$^2H_1$ and 2-OH-$^2H_1$[a]

	1*S*,1′*R*,3′*R* 1*R*,1′*S*,3′*S*	1*R*,1′*R*,3′*R* 1*S*,1′*S*,3′*S*
1-OH-$^2H_1$	0.913 ± 0.02	0.087 ± 0.004
2-OH-$^2H_1$	0.937 ± 0.03	0.063 ± 0.004
Average	0.925 ± 0.03	0.075 ± 0.004

[a] Error limits are standard deviations of repetitive scans. See Experimental Section.



alcohol (7-OH-$^2H_1$). The corresponding dihydro derivative yielded only dihydrosantolina alcohol (8-OH-$^2H_1$). The products were purified by GLC for analysis by NMR.

Stereochemistry of 6-OH-$^2H_1$, 7-OH-$^2H_1$, and 8-OH-$^2H_1$. Each of the three alcohols was a mixture of isotopic isomers. NMR spectra of the region where the vinyl moieties in 6-OH-$^2H_1$, 7-OH-$^2H_1$, and 8-OH-$^2H_1$ absorb are shown in Figure 2. Integration of the spectra showed that all of the deuterium is located in the terminal methylene groups. Upon irradiation at the deuterium resonance position, the NMR spectra of deuterated yomogi and artemisia alcohols exhibited a pair of AB quartets of unequal intensity in the vinyl region. The more intense AB quartet in yomogi alcohol results from the protons at 4.92 and 5.80 ppm coupled by 17.45 Hz. From the relative magnitudes of J_{AB}, we conclude that the vinyl hydrogens are trans in the more intense quartet and cis in the other. The ^1H NMR spectrum of artemisia alcohol also affords two AB quartets of unequal intensity. The larger (δ 4.97 and 5.82) had J_{AB} = 17.65 Hz, and the smaller (δ 4.96 and 5.82), J_{AB} = 10.85 Hz. Again the proton on the terminal methylene is mostly trans to the vicinal olefinic proton. The relative intensities of the AB quartets are listed in Table II.

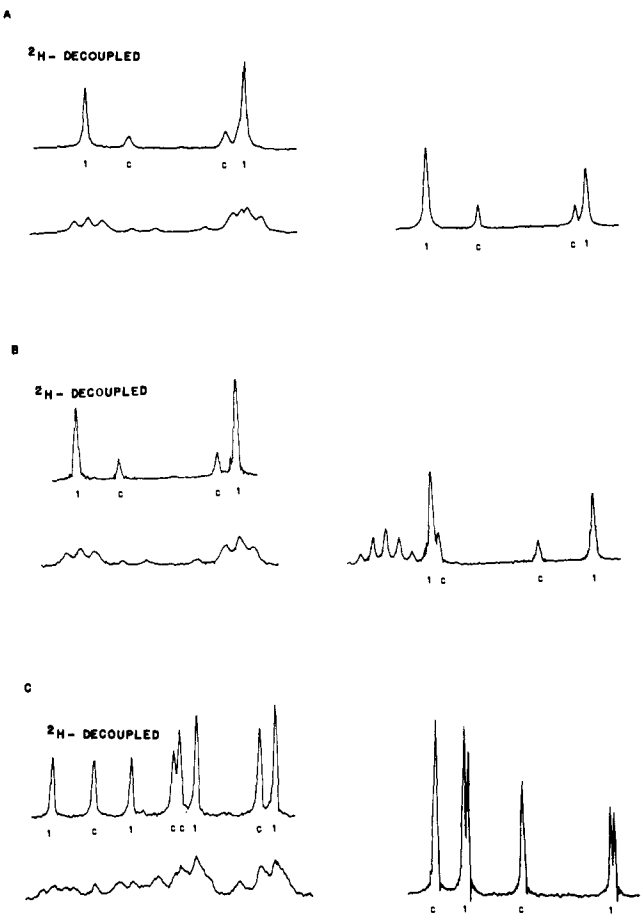


Figure 2. NMR spectra of the olefinic region for (A) **6-OH-$^2H_1$**, (B) **7-OH-$^2H_1$**, and (C) **8-OH-$^2H_1$**. Chemical shifts and coupling constants are tabulated in the Experimental Section.

Table II. ^2H Distribution in the Hydrolysis Products

Compd	^1H	Normalized intensity[a] ^1H ($J \simeq 17$ Hz)	Normalized intensity[a] ^1H ($J \simeq 10$ Hz)
6-OH-$^2H_1$	CHD=	0.792 ± 0.06	0.208 ± 0.02
	=CH-	0.800 ± 0.05	0.200 ± 0.03
7-OH-$^2H_1$	CHD=	0.797 ± 0.04	0.203 ± 0.02
	=CH-	0.798 ± 0.03	0.202 ± 0.01
8-OH-$^2H_1$	CHD=	0.518 ± 0.02	0.482 ± 0.02
	=CH-	0.508 ± 0.02	0.492 ± 0.01

[a] Determined from two independent runs by cutting and weighing three copies of three separate spectra for each alcohol. Error limits are reported as standard deviations. See Experimental Section.

In the absence of ^2H decoupling each of the lines in the AB quartets is split into a triplet. The geminal coupling constant between the two protons on the terminal methylene group is small, and as a result the upfield portion of each AB quartet is only slightly broadened. On the other hand, triplet patterns are clearly discernible in the low-field half of the AB spin system (see Figure 2).

The vinyl region of the NMR spectrum of dihydrosantolina alcohol is more complex due to two additional couplings introduced by the allylic proton. Two coupling patterns (marked t and c in Figure 2) of almost equal intensity (see Table II) are found. The pattern with the larger vicinal olefinic coupling constant is assigned to protons trans on the double bond (H_t) at δ 5.01 and 5.07. The high field proton is split into a doublet of doublets by the vicinal (J = 17.15 Hz) and allylic (J = 0.45 Hz) couplings, and the low field proton also appears as a doublet of doublets (J = 17.15 and 9.35 Hz). The remaining peaks are assigned to the cis protons (H_c) at 5.08 and 5.57 ppm. The high field proton appears as a doublet (J = 10.25 Hz) with the peaks only slightly broadened by homoallylic coupling, while the low-field pattern is a doublet of doublets (J = 10.25 and 9.35 Hz).

Table III. Comparison of Experimental and Calculated $J_{^1H-^2H}$'s in [1-^2H]Chrysanthemol, [1-^2H]Artemisia Alcohol, and [1-^2H]Yomogi Alcohol

Compd	Designation	J $^1H-^1H$[a]	J $^1H-^2H$(expt)[a]	J $^1H-^2H$(calcd)[b]
1-OH-$^2H_1$	1,1 (gem)	11.35	1.61	1.75
6-OH-$^2H_1$	6,7 (trans)	17.45	2.57	2.69
	6,7 (cis)	10.60	1.52	1.63
7-OH-$^2H_1$	6,7 (trans)	17.65	2.65	2.72
	6,7 (cis)	10.85	1.55	1.67

[a] Obtained on a Varian XL-100-15 at 50-Hz sweep width. [b] Calculated using $J_{^2H} = J_{^1H}(\gamma_{^2H}/\gamma_{^1H})$.[15]

Table IV. Stereochemistry of C(1) During Ionization of **1-OPyl-$^2H_1$** and **2-OPyl-$^2H_1$**

	$X_{\text{antiperiplanar}}$	$X_{\text{periplanar}}$
1-OPyl-$^2H_1$	0.860	0.140
2-OPyl-$^2H_1$	0.515	0.485

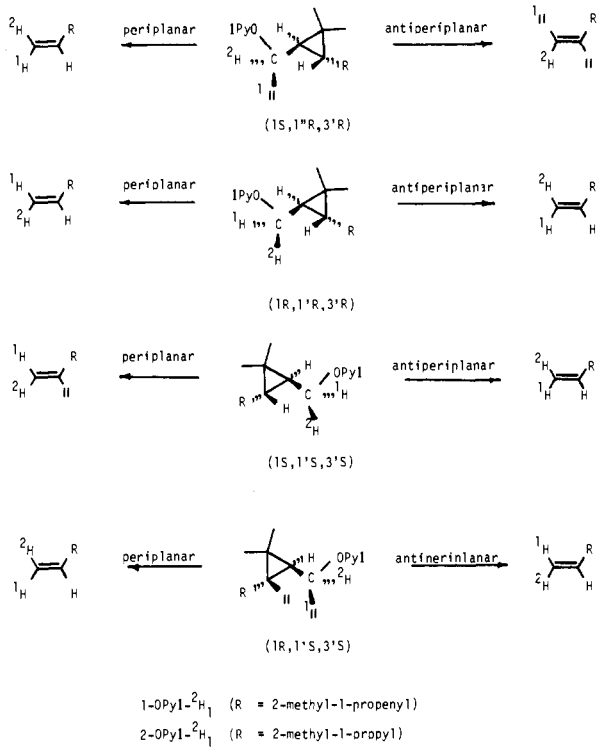
The cis and trans $^1H-^2H$ vicinal coupling constants can be determined from the undecoupled spectra of artemisia and yomogi alcohols (see Figure 2) and are listed in Table III along with the C(1) geminal $^1H-^2H$ coupling constant for [1-^2H]chrysanthemol. Since the magnitude of $J_{^1H,^2H}$ can be estimated by the equation[15]

$$J_{^2H} = J_{^1H}(\gamma_{^2H}/\gamma_{^1H}) \quad (1)$$

(where $\gamma_{^1H}$ and $\gamma_{^2H}$ are the magnetogyric ratios for hydrogen and deuterium), we have another check on our stereochemical assignments. As expected the protons at C(6) in both alcohols which have the larger $J_{^1H,^1H}$'s have larger $J_{^1H,^2H}$'s. It should be noted that J's calculated from eq 1 were from 3 to 9% larger than the corresponding values and the differences are outside of the uncertainties in our measurements.[16] The undecoupled spectrum of [2′-$^2H_1$]dihydrosantolina alcohol was not sufficiently well-resolved for us to measure $^1H-^2H$ couplings.

Since the precursors of alcohols **6-OH-$^2H_1$**, **7-OH-$^2H_1$**, and **8-OH-$^2H_1$** are mixtures of perhaps as many as four diastereomers, the data in Table II must be corrected for "mislabeling". Our NMR analysis of the stereochemistry at C(1) in **1-OPyl-$^2H_1$** and **2-OPyl-$^2H_1$** only permits us to distinguish between stereoisomers which are not enantiomers. Optical rotations demonstrate that the mixtures are mostly composed of the two diastereomers with the R absolute configuration at C(1′) and C(3′). However, the stereochemical mix of **1-OH-$^2H_1$** and **2-OH-$^2H_1$** may not exactly reflect the 98:2 ratio of enantiomers of aldehyde **5** used in the yeast alcohol dehydrogenase reduction. Scheme II shows the stereochemical consequence for ionization from periplanar and antiperiplanar conformations, with respect to C(1)–O and C(1′)–C(3′) bonds, in each of the four expected diastereomers of **1-OPyl-$^2H_1$** and **2-OPyl-$^2H_1$**. Since the enantiomers in each pair give the same relative configuration of ^1H, ^2H, H, and R in the products, the normalized intensities shown in Table II were used to calculate the mole fraction (X) of pyridinium iodide which ionizes from

Scheme II. Reactant and Product Stereochemistry for Hydrolysis of 1-OPyl-$^2H_1$ and 2-OPyl-$^2H_1$



the antiperiplanar conformation by solving the simultaneous equations shown below. The results are listed in Table IV.

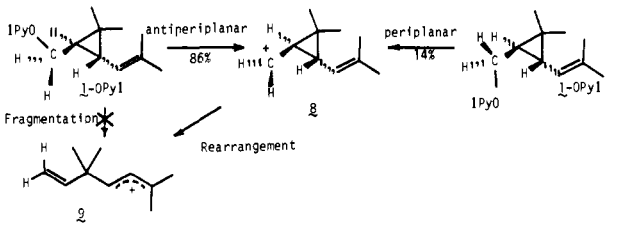
$$(X_{\text{antiperiplanar}})(X_{SRR+RSS}) + (X_{\text{periplanar}})(X_{RRR+SSS}) = X_{\text{trans}} \quad (2)$$

$$(X_{\text{antiperiplanar}})(X_{\text{RRR+SSS}}) + (X_{\text{periplanar}})(X_{SRR+RSS}) = X_{\text{cis}} \quad (3)$$

Influence of the Double Bond. From the NMR spectra shown in Figure 2, it is clear that the double bond at C(3′) influences the stereochemistry of the ionization step. If one uses dihydrochrysanthemylpyridinium iodide, where the ratio of ionization, antiperiplanar/periplanar, is 1.04 as a nonolefinic reference, the double bond accelerates ionization from the antiperiplanar conformation by ca. 6.0 relative to the periplanar arrangement. This corresponds to $\Delta\Delta G^{\ddagger} = 1.05$ kcal/mol at 25 °C for ionization from the two conformations. In comparison, replacing a trans hydrogen at C(2′) in the unsubstituted parent system by a methyl group (the *trans*-2′-methylcyclopropylcarbinyl system) gives an antiperiplanar/periplanar rate ratio of 3.1, corresponding to $\Delta\Delta G^{\ddagger} = 0.67$ kcal/mol at 25 °C.[9]

One would normally expect the stereoselectivity at C(1) to be directly proportional to the overall rate enhancement produced by a group attached to the cyclopropane ring. However, introducing a double bond between C(1″) and C(2″) in dihydrochrysanthemyloxypyridinium iodide increases the rate of solvolysis by only threefold, while *trans*-2′-methylcyclopropylcarbinyl mesylate solvolyzes 14.4 times faster than the parent mesylate. We suggest that rate comparisons for **1-OPyl** and **2-OPyl** are misleading and should not be used as a measure of conjugative interaction[8] between a cyclopropylcarbinyl cation and a directly attached double bond. Double bonds are dichotomous with respect to their influence on the stability of carbonium ions. Normally the stabilizing conjugative interaction dominates, but in systems where conjugation between the double bond and neighboring positive centers is not possible, the destabilizing inductive effect is considerable, producing rate retardations of up to 10^{-4}.[19] Inductive and conjugative contributions to the observed rate almost cancel for the chrysanthemyl cation because conjugation between the cyclopropane ring and the double bond is reduced by poor orbital overlap.[8] Although conjugation between the two groups is revealed by a preference for antiperiplanar ionization, there is no reliable method for separating the effect of conjugation and induction on the solvolysis rate.

In theory two cations can be generated directly from **1-OPyl** upon ionization. However, we have shown that the diastereo-



topic protons at C(1) in **1-OPyl** are scrambled to the same extent in products derived from allylic cation **9** (86:14) and head-to-head products formed after cyclopropylcarbinyl-cyclopropylcarbinyl rearrangement of **8** (84:16).[20] These observations suggest that **1-OPyl** does not fragment directly to **9** but is formed from cyclopropylcarbinyl cation **8** by a facile fragmentation.

Finally, we have shown that binding forces between the substrate and the enzyme must be responsible for enforcing stereospecificity in a reaction that is normally stereoselective in the absence of enzyme. The difference in rates for ionization from periplanar and antiperiplanar conformers is too small to suggest that this kinetic differential is responsible for selection of one conformer over another during evolution of the enzymatic reaction. Rather, we suggest that an antiperiplanar alignment of presqualene and prephytoene pyrophosphate in the enzyme–substrate complex is essential for facilitating the regiospecific rearrangements that occur after ionization.[5]

Experimental Section

General. Melting points were obtained on a Fisher–Johns melting point stage or in open capillaries in a Thomas–Hoover melting point apparatus and are uncorrected. Carbon–hydrogen microanalyses were performed by Chemalytics, Inc., Tempe, Arizona.

Nuclear magnetic resonance (NMR) spectra were obtained on Varian Associates A-60, EM-360, EM-390, and XL-100 spectrometers and are reported in parts per million (δ, ppm) downfield from tetramethylsilane (Me_4Si) internal standard. Unless otherwise indicated, NMR spectra were obtained in carbon tetrachloride (Mallinkrodt, SpectrAR grade). NMR solvent giving equally intense Me_4Si and chloroform resonances of amplitude suitable for use with 10% solutions was prepared by adding 0.5 mL (0.32 g, 3.68 mmol, 44.2 mmol of protons) of Me_4Si and 5.30 g (44.2 mmol) of chloroform to 50 mL of carbon tetrachloride. Spectra were corrected when necessary for nonlinearity using the 436-Hz (at 60 MHz) singlet resonance of chloroform. Infrared (IR) spectra were obtained on a Beckman IR-5 spectrophotometer. Optical rotations were measured using a Perkin-Elmer Model 141 digital polarimeter operating at 589 nm (sodium D line), employing the specified solvent and a 1-dm water-jacketed cell thermostated to 25 ± 0.1 °C. Optical rotations are reported as specific rotations ($[\alpha]^{25}_D$) and concentrations in grams per 100 mL of solvent, e.g., (*c* 5, $CHCl_3$).

Analytical gas–liquid chromatography was performed on a Varian-Aerograph Model 1200 instrument equipped with a flame ionization detector and an Autolab Model 6300 digital integrator. Repetitive injections showed that the system's reproducibility averaged ±0.5% and never exceeded ±2%. Analytical separations were achieved using 500 ft × 0.03 in. (i.d.) stainless steel open tubular columns utilizing the stationary phases Carbowax 20M (recrystallized from ethanol), 95:5 OV-101/Igepal CO-880 (both obtained from Analabs, Inc.), or SF-96 (viscosity 50 cSt at 25 °C; Analabs, Inc.) as indicated and operated at the specified temperature at a nitrogen flow rate of 10 mL/min.

Preparative gas–liquid chromatographic separations were performed on a Varian-Aerograph Model A90-P3 thermal conductivity instrument, using 12 ft × ¼ in. (o.d.) glass columns packed with 10% Carbowax 20M on 60/80 mesh Anakrom ABS diatomaceous earth support, or on a 3 ft × ¼ in. (o.d.) stainless steel column packed with 95:5 OV-101/Igepal CO-880 on 50/60 mesh Anakrom ABS.

Thin-layer chromatography was carried out on 75 mm × 25 mm "Bakerflex" precoated silica gel 1B-F sheets (silica gel G with fluorescer; J. T. Baker Co.) in vapor-saturated tanks, visualizing the developed spots first with 254-nm light and then with iodine staining.

(1′*R*,3′*R*)-[1-$^2H_2$]-2′,2′-Dimethyl-3′-(2″-methyl-1″-propenyl)cyclopropylmethanol, (1′*R*,3′*R*)-[1-$^2H_2$]Chrysanthemol (1-OH-$^2H_2$). To a stirred suspension of 2.0 g (47.7 mmol) of lithium aluminum deuteride (99% d_4, Brinkmann) in 100 mL of anhydrous ether was added 12.0 g (66 mmol) of methyl (1*R*,3*R*)-chrysanthemate[5a] in 100 mL of anhydrous ether over 1 h. The reaction mixture was brought to reflux and allowed to stir overnight before excess hydride was decomposed and the salts were hydrolyzed by dropwise addition of saturated aqueous ammonium chloride solution until the inorganic material precipitated. The clear ether layer was decanted and the precipitate washed several times with ether. The combined ethereal filtrates were dried over anhydrous magnesium sulfate and solvent was removed at reduced pressure to give 9.50 g (92%) of a faintly-colored oil which was distilled, bp 74–76 °C (4 mm), before use in the next step: NMR (CCl_4) 0.58–1.26 (2, m, H at C(1) and C(3)), 0.97 and 1.07 (6, pr of s, methyls at C(2′)), 1.59 (6, d, allylic methyls), 3.75 (1, br s, hydroxyl proton), and 4.62 ppm (1, d of septets, ^3J = 8 Hz, ^4J = 1.5 Hz, olefinic proton); IR (neat) 3250, 2900, 2850, 2700, 2180, 2080, 1640, 1445, 1370, 1270, 1185, 1140, 1085, and 960 cm^{-1}.

(1′*R*,3′*R*)-[1-^2H]-2′,2′-Dimethyl-3′-(2″-methyl-1″-propenyl)cyclopropylmethanal, (1′*R*,3′*R*)-[1-^2H]Chrysanthemal, (5-^2H). Collins reagent[21] was prepared from 49.0 g (0.491 mol) of chromium trioxide (dried in vacuo over phosphorus pentoxide) and 77.5 g (0.983 mol) of pyridine (dried over 3Å molecular sieves) in 400 mL of dry dichloromethane and allowed to stir for 45 min before dropwise addition of 10.8 g (0.069 mol, sevenfold excess of Collins reagent) of (1′*R*,3′*R*)-[1-$^2H_2$]chrysanthemol in 60 mL of dry dichloromethane over 45 min. The reaction mixture was allowed to stir for 2 h at room temperature, during which time large amounts of a tarry precipitate were formed. The dichloromethane solution was decanted, the tarry salts were washed several times with ether, and the combined organic layers washed in succession with 10% aqueous sodium hydroxide, 10% aqueous hydrochloric acid, and water. The organic layers were dried over anhydrous magnesium sulfate and solvent was removed at reduced pressure, giving 7.52 g (0.049 mol, 71%) of a faintly-colored oil which was more than 97% pure by GLC with no starting material remaining. Earlier attempts at further purification resulted in partial isomerization to the undesired cis isomer, so this material was used immediately in the next step of the synthetic sequence: NMR (CCl_4) 1.15 and 1.25 (6, s, CH_3 at C(2)), 1.56 (1, d of d, H at C(1′), $J_{1′,3′}$ = 6.0 Hz), 1.67 (6, d, allylic CH_3, J = 1.7 Hz), 2.16 (1, d of d, H at C(3′), $J_{3′,1″}$ = 7.5 Hz), and 4.79 ppm (1, d of septets, olefinic H); NMR is identical with that of (1′*R*,3′*R*)-chrysanthemal except for lack of 9.34-ppm doublet and a C(1),C(1′) coupling; IR (CCl_4) 3000, 2960, 2910, 2860, 2710, 2060, 1680, 1480, and 1110 cm^{-1}.

(1*S*,1′*R*,3′*R*)-[1-^2H]-2′,2′-Dimethyl-3′-(2″-methyl-1″-propenyl)-cyclopropylmethanol, (1*S*,1′*R*,3′*R*)-[1-^2H]Chrysanthemol (1-OH-$^2H_1$). Fleischmann's baker's yeast (316 g) and 400 g of sucrose were added to 2500 mL of distilled water held at 35 °C.[12] Fermentation was monitored by CO_2 evolution and foaming was controlled by frequent swirling and by the addition of a few drops of *n*-octanol. After 30 min, 7.52 g (0.049 mol) of (1′*R*,3′*R*)-[1-^2H]chrysanthemal in 20 mL of ethanol was added, and the fermentation allowed to proceed for 18 h, by which time CO_2 evolution had ceased. The reaction mixture was steam-distilled until no more of the desired product could be detected in the distillate by GLC. The product-containing distillate was saturated with ammonium chloride and then extracted with pentane. The combined organic layers were washed with water, dried over anhydrous magnesium sulfate, and solvent was removed at reduced pressure. The residue was distilled through a 10-cm Vigreux column, giving two fractions: 2.40 g (bp 69–72 °C (2.8 mm)), 73% pure by GLC; and 2.78 g (bp 73–75 °C, (2.8 mm)), 95% pure by GLC. Analytical samples were collected by preparative GLC; NMR (CCl_4) 0.60–1.28 (2, m, H at C(1′) and C(3′)), 1.01 and 1.10 (6, two s, methyls at C(2′)), 1.62 (6, d, allylic methyls), 3.32 (1, d of t, $J_{1,1′}$ = 8.3 Hz, $J_{1H,2H}$ = 1.51 Hz, H at C(1)), 3.73 (1, br s, hydroxyl), and 4.75 ppm (1, d of septets, ^3J = 7.5 Hz, ^4J = 1.5 Hz, olefinic H); IR (CCl_4) 3650, 3350, 2900, 2300, 1450, 1390, 1270, 1195, 1160, 1125, 1040, 985, 965, and 930 cm^{-1}.

(1*S*,1′*R*,3′*R*)-[1-^2H]-2′,2′-Dimethyl-3′-(2″-methyl-1″-propyl)cyclopropylmethanol, (1*S*,1′*R*,3′*R*)-[1-^2H]Dihydrochrysanthemol (2-OH-$^2H_1$). The (1*S*,1′*R*,3′*R*)-[1-^2H]chrysanthemol fraction (bp 69–72 °C (2.8 mm), 2.40 g, 15.5 mmol, containing 73% of the desired alcohol) was dissolved in 100 mL of absolute ethanol and hydrogenated at 40 psi of hydrogen over Adams catalyst in a Parr shaker. After 12 h, the solution was degassed and gravity filtered. GLC analysis (500 ft Carbowax 20M, 140 °C) showed reduction to be complete. The filtrate was added to an equal volume of water and extracted three times with pentane. The combined organic layers were dried over anhydrous magnesium sulfate and solvent was removed at reduced pressure to afford 2.30 g (94% crude) of a colorless oil. Short-path distillation at atmospheric pressure (640 mm) removed low-boiling impurities and resulted in an alcohol which was 90% pure (1.796 g, 11.8 mmol, 74%). Analytical samples were collected by GLC: NMR (CCl_4) 0.16–0.53 (2, m, H at C(1′) and C(3′)), 0.83 and 0.89 (6, two d, J = 1 Hz, CH_3's at C(2′)), 1.01 (6, d, ^3J = 2.5 Hz, CH_3's at C(2″)), 1.06–1.81 (3, m, aliphatic H at C(1″) and C(2″)), 2.27 (1, s, hydroxyl H), and 3.32 ppm (1, d of t, $J_{1,1′}$ = 8.35 Hz, $J_{1H,2H}$ = 1.6 Hz, *pro-R* proton at C(1)); IR (CCl_4) 3650, 3350, 2900, 2150, 1470, 1380, 1370, 1160, 1130, 1070, and 970 cm^{-1}.

4-[(1*S*,1′*R*,3′*R*)-[1-^2H]-2′,2′-Dimethyl-3′-(2″-methyl-1″-propenyl)cyclopropylmethoxy]pyridine, 4-[(1*S*,1′*R*,3′*R*)-[1-^2H]Chrysanthemyloxy]pyridine (10-$^2H_1$). Sodium hydride (0.491 g, 20.5 mmol, obtained from an oil dispersion) was added to 20 mL of dry dimethyl sulfoxide (Me_2SO) and the stirred slurry was heated to 70 °C for 90 min. A 2.02-g portion (13.05 mmol, 57% excess of sodium hydride) of (1*S*,1′*R*,3′*R*)-[1-^2H]chrysanthemol in 15 mL of dry Me_2SO was added over 10 min, and the resulting green-brown suspension allowed to stir for 2 h at 70 °C. 4-Chloropyridine[22] (1 equiv, 1.498 g, 13.2 mmol) in 10 mL of dry Me_2SO was added over 10 min, and the reaction mixture was allowed to stir at 70 °C overnight. The reaction mixture was cooled to room temperature and poured into 100 mL of distilled water, and the resulting cloudy solution was extracted with pentane. The combined organic layers were washed with water, dried over anhydrous magnesium sulfate, and solvent was removed at reduced pressure to afford 2.57 g (11.1 mmol, 85%) of a yellow oil: NMR (CCl_4) 0.65–1.43 (2, m, H at C(1′) and C(3′)), 1.03 and 1.10 (6, s, CH_3's at C(2′)), 1.60 (6, m, allylic CH_3's), 3.72 (1, d of t, H at C(1), ^3J = 8.5 Hz, $J_{1H,2H}$ = 2 Hz), 4.70 (1, d of septets, olefinic H, ^3J = 7.5 Hz, ^4J = 1.5 Hz), 6.49 (2, m, H at C(3) and C(5) of pyridine ring), and 8.03 ppm (2, m, H at C(2) and C(6) of pyridine ring); IR (neat) 2850–3000, 1590, 1570, 1500, 1380, 1285, 1210, 1130, 1030, 995, 970, 835, and 815 cm^{-1}.

4-[(1*S*,1′*R*,3′*R*)-[1-^2H]-2′,2′-Dimethyl-3′-(2″-methyl-1″-propyl)cyclopropylmethoxy]pyridine, 4-[(1*S*,1′*R*,3′*R*)-[1-^2H]Dihydrochrysanthemyloxy]pyridine (11-$^2H_1$). Following the procedure described for 10-$^2H_1$, 0.384 g (16.0 mmol, prepared from 0.628 g of oil dispersion) of sodium hydride was stirred with 15 mL of dry Me_2SO for 90 min at 70 °C, and then 1.796 g (11.4 mmol) of (1*S*,1′*R*,3′*R*)-[1-^2H]dihydrochrysanthemol in 10 mL of dry Me_2SO was added over 15 min. The dark brown mixture was allowed to react at 70 °C for 3 h before 1.324 g (11.7 mmol, 2% excess) of freshly prepared 4-chloropyridine in 10 mL of dry Me_2SO was added, and the ensuing chocolate-colored solution was allowed to stir at 70 °C overnight. Workup as previously described gave 2.365 g (10.1 mmol, 88%) of a clear golden oil; NMR (CCl_4) 0.25–1.87 (11, m, aliphatic and cyclopropyl H), 1.00 (6, s, CH_3's at C(2″)), 3.61 (1, d of t, H at C(1), ^3J = 7 Hz), 6.28 (2, d of d, H at C(3) and C(5) of pyridine ring), and 7.84 ppm (2, m, H at C(2) and C(6) of pyridine ring); IR (neat) 2900, 1590, 1570, 1500, 1480, 1280, 1205, 1025, 995, 975, and 815 cm^{-1}.

***N*-Methyl-4-[(1*S*,1′*R*,3′*R*)-[1-^2H]-2′,2′-dimethyl-3′-(2″-methyl-1″-propenyl)cyclopropylmethoxy]pyridinium Iodide, *N*-Methyl-4-[(1*S*,1′*R*,3′*R*)-[1-^2H]chrysanthemyloxy]pyridinium Iodide (1-OPyI-$^2H_1$).** In a typical preparation, 0.266 g (1.14 mmol) of neat alkoxypyridine was treated with 0.244 g (1.72 mmol, 1.5 equiv) of iodomethane at room temperature. Within 30 min the reaction mixture had solidified to a bright yellow crystalline mass which was then allowed to stand at −10 °C for 24 h. Recrystallization from ethyl acetate/pentane with *slow* cooling to 0 °C afforded a first crop of off-white powder, mp 115–116 °C (110 mg, 25%). The pyridinium iodide decomposed slowly at room temperature and satisfactory carbon–

hydrogen analyses could not be obtained; however, no decomposition was detected over a 1-month period when the material was stored at −10 °C. Subsequent recrystallization attempts isolated smaller amounts of product and resulted in yellowing of the mother liquor. Some unmethylated starting material was detected by thin-layer chromatography on silica gel developed with 10:1 chloroform/2-propanol. Successive recrystallization resulted in concentration of impurities, causing difficulty in obtaining solid material after two crops; total yield was 220 mg (52%): NMR ($CDCl_3$) 0.8–1.5 (2, m, H at C(1′) and C(3′)), 1.13 and 1.20 (6, s, CH_3's at C(2′)), 1.73 (6, m, allylic methyls), 4.33 (1, d of t, ^3J = 8.5 Hz, $J_{1H,2H}$ = 1 Hz, H at C(1)), 4.55 (3, s, *N*-methyl), 4.95 (1, d of septets, olefinic H), 7.45–8.46 (2, m, H at C(3) and C(5) of pyridine ring, ^3J = 7.5 Hz), and 9.25–9.48 ppm (2, m, H at C(2) and C(6) of pyridine ring); IR (KBr) 2850, 1640, 1580, 1520, 1395 (br), 1330, 1210, 1185, 860, 845, and 700 (v br) cm^{-1}.

***N*-Methyl-4-[(1*S*,1′*R*,3′*R*)-[1-^{2}H]-2′,2′-dimethyl-3′-(2″-methyl-1″-propyl)cyclopropylmethoxy]pyridinium Iodide, *N*-Methyl-4-[(1*S*,1′*R*,3′*R*)-[1-^{2}H]dihydrochrysanthemyloxy]pyridinium Iodide (2-OPyI-$^2H_1$).** A 0.500-g portion (2.14 mmol) of 4-[(1*S*,1′*R*,3′*R*)-[1-^{2}H]dihydrochrysanthemyloxy]pyridine was allowed to react with 0.61 g (4.28 mmol, 2 equiv) of iodomethane in pentane at reflux for 1 h. Sufficient ethyl acetate was then added to dissolve the resulting brown oil, and the reaction mixture was cooled to −10 °C overnight in an unsuccessful attempt to induce crystallization. Solvent was removed at reduced pressure to give 0.68 g (85%) of a brown oil which successfully resisted all attempts at crystallization in numerous solvent systems. Thin-layer chromatography (silica gel, 10:1 chloroform/2-propanol) revealed small amounts of residual starting materials (both alcohol and alkoxypyridine) which may account for the difficulty in crystallizing the salt. The NMR spectrum of the salt showed less than 8% contamination by unreacted starting materials, and this material was used in the solvolysis experiments without further purification; NMR ($CDCl_3$) 0.36–1.93 (5, m, aliphatic and cyclopropyl H), 0.89 and 0.98 (6, pr of s, CH_3's at C(2′)), 1.13 (6, d, CH_3's at C(2″, ^4J = 2 Hz), 4.16 (1, d of t, H at C(1), ^3J = 7 Hz, $^2J_{1H,2H}$ = 2 Hz), 4.55 (3, s, *N*-methyl), 7.55 (2, m, H at C(3) and C(5) of pyridine ring), and 9.23 ppm (2, d, H at C(2) and C(6) of pyridine ring).

Solvolysis. In a typical preparative scale solvolysis, 0.250 g (0.67 mmol) of pyridinium iodide was dissolved in 50 mL of distilled water containing at least a tenfold molar excess of sodium bicarbonate, and the solution was allowed to react at 25 °C for approximately 10 half-lives (99.99% reaction). The clear aqueous solution was saturated with sodium chloride and extracted several times with pentane. The combined organic layers were dried over anhydrous magnesium sulfate and the product concentrated to ca. 1 mL at reduced pressure. The products were analyzed by GLC (generally 500 ft Carbowax 20M near 140 °C) and then collected by preparative GLC (generally 12 ft Carbowax 20M, 100–150 °C).

In the case of **1-OpyI-$^2H_1$**, GLC analysis revealed two major products: [^{2}H]yomogi alcohol, 76% and [^{2}H]artemisia alcohol, 18%. The labeled *dihydro* derivative **2-OPyI-$^2H_1$** gave a single product: [^{2}H]dihydrosantolina alcohol (**8-OH-$^2H_1$**), ≥99% pure. Contaminants in the starting material were stable during the solvolysis. Control experiments also demonstrated that the products were stable to the reaction conditions.

***trans*-[7-^{2}H]-2,5,5-Trimethylhepta-3,6-dien-2-ol, [7-^{2}H]Yomogi Alcohol (6-OH-$^2H_1$).** An analytically pure sample gave an NMR spectrum identical with that previously reported[23] except for the deuterium-coupled resonances of the protons at C(6) and C(7): NMR (CCl_4, ^{2}H-decoupled) 1.10 (6, s, methyls at C(2)), 1.25 (6, s, methyls at C(5)), 1.68 (1, s, hydroxyl), 4.94 (1, d of d, H at C(1), $^3J_{cis}$ = 10.60 Hz, $^3J_{trans}$ = 17.45 Hz), 5.50 and 5.63 (2, AB q, H at C(3) and C(4), $^3J_{trans}$ = 16.10 Hz), and 5.83 ppm (1, d of d, H at C(6)); IR (CCl_4) 3600, 3350, 2950, 2250, 1620, 1460, 1380, 1360, 1320, 1290, 1230, 1190, 980, 910, and 840 cm^{-1}.

[7-^{2}H]-2,5,5-Trimethylhepta-2,6-dien-4-ol, [7-^{2}H]Artemisia Alcohol (7-OH-$^2H_1$). The GLC-collected sample gave an NMR spectrum identical with that obtained for the nondeuterated molecule[5a] (except for the protons at C(6) and C(7)); NMR (CCl_4, ^{2}H-decoupled) 0.97 and 1.00 (5, s, methyls at C(5)), 1.70 and 1.75 (6, d, allylic methyls), 3.95 (1, d, H at C(4), ^3J = 9.20 Hz), 4.98 (1, d of d, H at C(7), $^3J_{trans}$ = 17.60 Hz, $^3J_{cis}$ = 10.85 Hz), 5.70 (1, d of septets, H at C(3), ^3J = 9.20 Hz, ^4J = 1.45 Hz), and 5.90 ppm (1, d of d, H at C(6)); IR (CCl_4) 3600, 3500, 2950, 2260, 1680, 1620, 1470, 1450, 1380, 1370, 1320, 1280, 1265, 1185, 1140, 1105, 990, 930, 890, and 840 cm^{-1}.

[2′-^{2}H]-2,5-Dimethyl-3-(ethenyl)hexan-2-ol, [2′-^{2}H]Dihydrosantolina Alcohol (8-OH-$^2H_1$). The GLC-collected sample gave an NMR spectrum identical (except for the olefinic region) with the reported spectrum:[5a] NMR (CCl_4, ^{2}H-decoupled) 0.88 (6, m, methyls at C(5)), 1.10 (6, s, methyls at C(2)), 1.15–1.75 (3, m, H at C(4) and C(5)), 1.70 (1, br, hydroxyl), 1.86–2.16 (1, m, H at C(3)), 5.01 (0.52, d of d, $^3J_{trans}$ = 17.15 Hz, $J_{2,3}$ = 0.45 Hz, *trans*-H at C(2′)), 5.08 (0.48, d, $^3J_{cis}$ = 10.25 Hz, *cis*-H at C(2′)), and 5.57 ppm (1, d of [d of d], $J_{1',3}$ = 9.35 Hz, H at C(1′)); IR (CCl_4) 3425, 2925, 2250, 1620, 1460, 1370, 1330, 1205, 1170, 1130, 1105, 990, 940, 910, 870, and 840 cm^{-1}.

Integration of NMR Spectra. Relative peak areas were determined from three separate scans of the appropriate olefinic regions for **6-OH**, **7-OH**, and **8-OH** using a 50-Hz sweep width. Three photocopies were made of each scan and the peaks were cut and weighed. The averages of the nine determinations were used to calculate relative areas. Standard deviations were calculated using a Hewlett-Packard Model 9810A calculator and the HP-V-1 library program "Statistics of a Single Variable". The results are presented in Tables I and II.

References and Notes

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