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Synthesis of (4*R*)- and (4*S*)-[4-$^2H_1$] Geraniol

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The pinene synthase (cyclase) from common sage (*Salvia officinalis*) catalyzes the conversion of geranyl pyrophosphate to the bicyclic olefins (+)-α-pinene and (+)-β-pinene (cyclaseIII), in addition to smaller amounts of monocyclic and acyclic monoterpene olefins. To obtain conclusive evidence concerning the stereospecificity of deprotonation in the biosynthesis of (+)- and (-)-α-pinene, we synthesized geraniol labeled stereospecifically with deuterium at C-4.

Key Words: Pinene, Geraniol, Stereospecificity, Monoterpene, Enantiomer.

INTRODUCTION

The biosynthesis of the commonly co-occurring α-pinene and β-pinene have been a subject of long-standing interest[1]. One main concern has been on the mechanism of the formation of the two structural isomers. This is solved by observing isotopically sensitive branching during the competitive formation of (-)-α-pinene and β-pinene in deuterium labeling experiments[2,3] and ^{2}H NMR-na spectra[4] as described previously.

A general stereochemical model for the coupled isomerization and subsequent cyclization of GPP to the monoterpenes has been proposed[5]. This scheme, as applied to the pinene synthases (**Scheme-I**), posits the initial ionization of GPP[1], with suprafacial migration of the pyrophosphate moiety of the resulting ion pair[2], to afford the enzyme-bound linalyl pyrophosphate intermediate[3]. Rotation about the newly generated C2-C3 single bond to the *cisoid* conformer overcomes the original impediment to the direct cyclization of the geranyl substrate, while subsequent ionization of this tertiary allylic isomer promotes C1-C6 cyclization of the *anti, endo*-form to the α-terpinyl cation[6]. A second electrophilic cyclization involving the remaining double bond of **6** gives rise to the pinyl cation[7] from which the indicated deprotonations lead to the pinenes. The bornyl cation[8] formed both by direct cyclization of **6** and by rearrangement of **9**, undergoes rearrangement to the camphyl cation[9] which yields camphene[10] upon deprotonation. The acyclic olefin[5] and the monocyclic olefins[11,12] are derived by deprotonation of **2** and/or **4** and **6**, respectively.

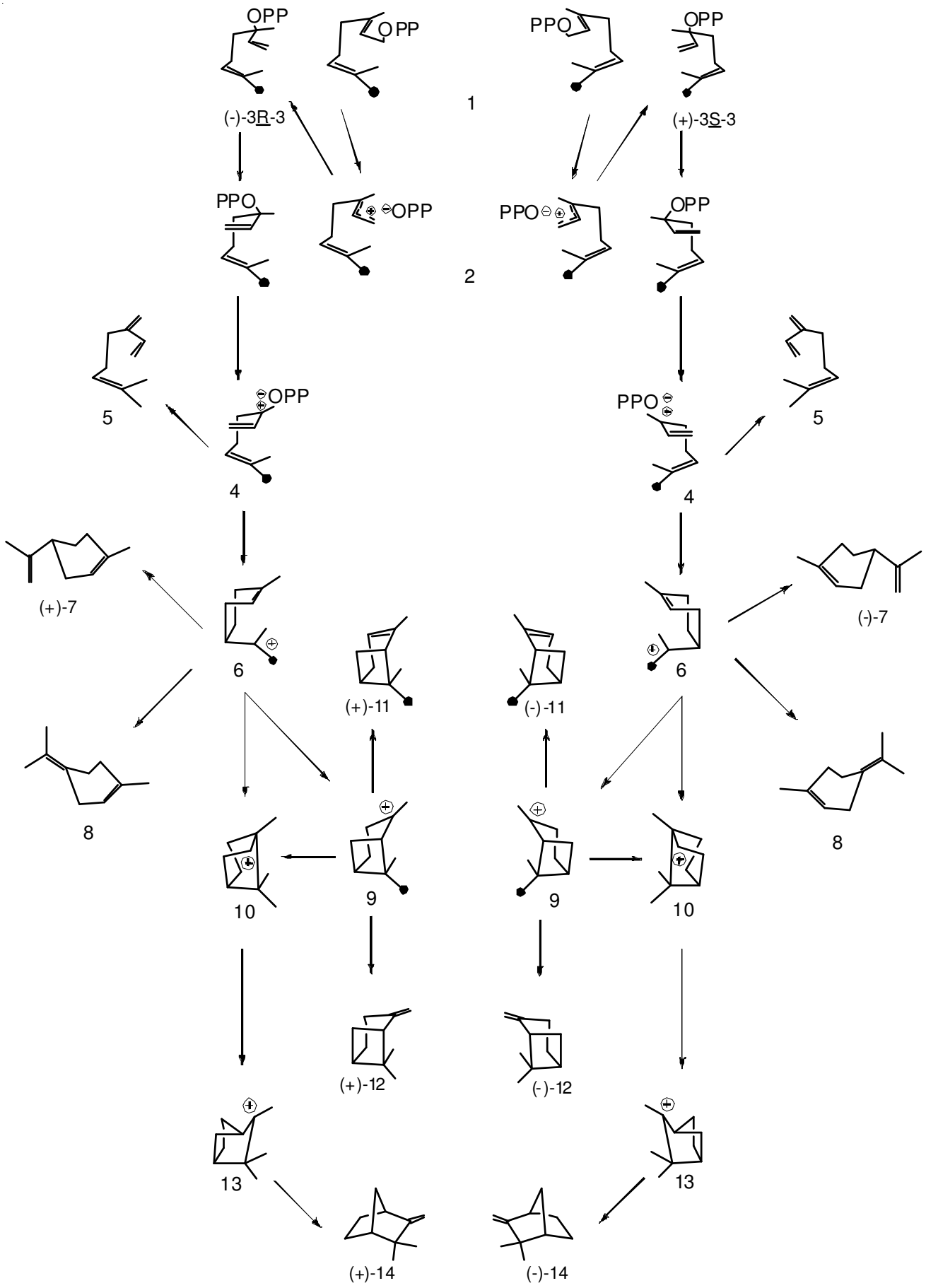
The stereochemistry of the enzymatic cyclizations of (1*R*)- and (1*S*)-[1-^{3}H, 2-^{14}C]GPP to the (+)-pinene and (-)-pinene have been examined[6] as has the enantioselectivity in the conversion of (+)-(3*R*)- and (-)-(3*S*)-[1-3H] linalyl pyrophosphate to these antipodal olefins[11]. The stereochemistry of the cyclizations which form the prochiral *gem*-dimethyl bridge of the α-pinene enantiomers have also been studied, using (6*E*)-[8-^{3}H]GPP as substrate and it was shown that the initial *anti*, *endo*-cyclization of (3*R*)- and (3*S*)-linaly pyrophosphates[3] are followed by the corresponding least-motion cyclization of the α-terpinyl intermediates[6] to form the cyclobutane rings of the pinenes[12]. All of these results are consistent with the mirror-image configuration outlined involving antipodal linalyl[4], α-terpinyl[12] and pinyl[7] carbocations.

Although considerable evidence supporting the competitive formation of (-)-α-pinene and (-)-β-pinene have been accumulated, the stereochemistry of the deprotonation step in the biosynthesis of α-pinene (α-4) has not been established conclusively. To obtain conclusive evidence concerning the stereospecificity of deprotonation in the biosynthesis of (+)-pinene and (-)-α-pinene, we synthesized geraniol labeled stereospecifically with deuterium at C-4. The optically active samples of geraniol were pyrophosphorylated at Washington State University and the deuterated GPP samples were incubated with the pinene cyclases.

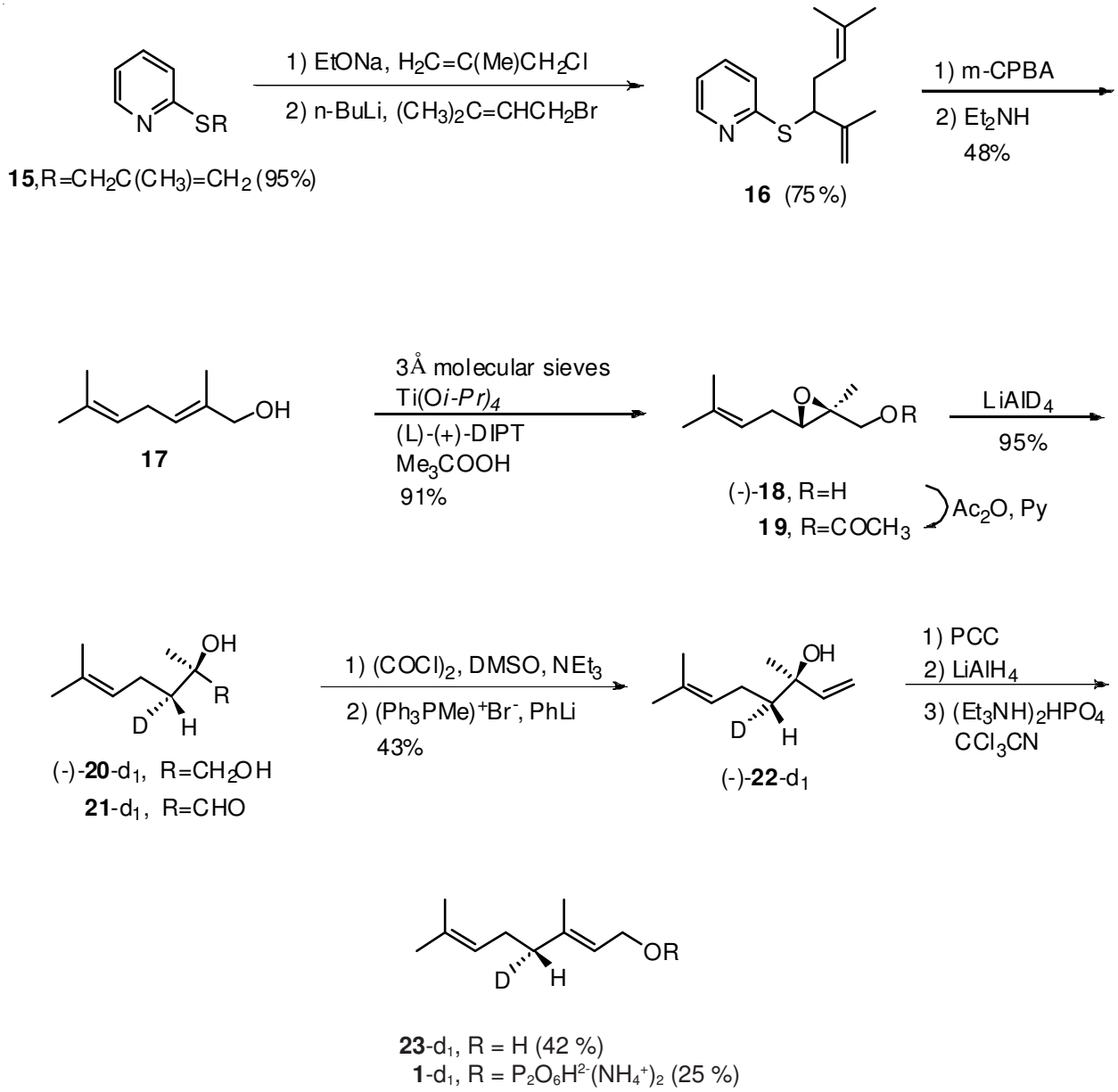
Now we report that the synthesis of (4*R*)- and (4*S*)-[4-$^2H_1$]GPP{(4*R*)- and (4*S*)-[4-$^2H_1$]} as outlined in **Scheme-II** for the (4R)-enantiomer.

EXPERIMENTAL

(3*RS*)-(±)-2,6-Dimethyl-3-(2-pyridylthio)-1,5-heptadine 16[7]: Yield (after distillation), 28.1 g (75 %); bp 110-118 (0.3



Scheme-I. Stereochemical model for the formation of monoterpene olefins by the various pinene synthases of sage. OPP denotes the pyrophosphate moiety



Scheme-II. Synthesis of (4*R*)- and (4*S*)-[4-$^2H_1$]GPP{(4*R*)- and (4*S*)-[4-$^2H_1$]}

mm) [lit. bp 110-120 ºC (1 mm)[7]]. (2*E*)-2.6-Dimethyl-2,5-heptadine-1-ol 17[7]; yield, 8.1 g (48 %); bp 100 - 103 ºC (9.5 mm) [lit. bp 97 - 99 ºC (9 mm)[7,8]]. ^{13}C NMR, δ 13.6, 17.6, 25.6, 26.6, 68.7, 122.4, 124.9, 131.9, 134.5.

(2*S*, 3*S*)-(-)-2,3-Epoxy-2,6-dimethyl-5-heptadine-1-ol ((-)-18)[1]: Yield, 3.03 g (91 %) of (-)-**18** ; $[\alpha]_D^{28}$ -12.7º (c 2.13, $CHCl_3$ [lit.$[\alpha]_D$ for the enantiomer, +12.6 ° (c 2.13, $CHCl_3$)[7]]. (2*S*, 3*S*)-**19** : IR (neat) ν_{max} 2973 (CH), 2932 (CH), 1746 (C=O), 1447 (C=C) cm^{-1} ; ^{1}H NMR (C_6D_6) δ1.13 (s, 3H, CH_3, 1.44 (s, 3H, $=CCH_3$), 1.59 (s, 3H, $=CCH_3$), 1.60 (s, 3H, CH_3CO), 2.05 and 2.21 (2 × 5-lines dt, 2H, *J* = 14.7 Hz, $=CHCH_2$-), 2.72 (t, 1H, *J* = 6.4 Hz, CHO), 3.81 (d, lH, *J* = 11.8 Hz, CH_2O), 4.10 (d, lH, *J* = 11.8 Hz, CH_2O), 5.14 (t, lH, *J* = 7.2 Hz, =CH-).

(2*R*, 3*R*)-(+)-2,3-Epoxy-2,6-dimethyl-5-heptadine-1-ol ((+)-18)[13]: Yield, 918 mg (74 %); $[\alpha]_D^{26}$ +13.2º (c 2.13, $CHCl_3$) [lit $[\alpha]_D$ +2.16º (c 2.13, $CHCl_3$)[9]]; 94 % ee;

(2*R*)-(-)-2,6-Dimethy1-5-heptadine-I,2-ol ((+)-20): (-)-20 : $[\alpha]_D^{28}$ -0.75º (c 1.00, $CHCl_3$)[lit $[\alpha]_D^{23}$ -6.7º (c 0.39, $CHCl_3$) for 60 % enantiomeric purity[9]] ; IR (neat) ν_{max} 3326 (OH), 2971 (CH), 1453 (C=C) cm^{-1}; ^{1}H NMR δ 1.19 (s, 3H, CH_3), 1.52 (m, 2H, -CH_2C), 1.63 (s, 3H, $=CCH_3$), 1.69 (s, 3H, $=CCH_3$), 1.94 (br, 2H, OH), 2.05 (m, 2H, $=CHCH_2$), 3.39 (d, lH, *J* = 10.9 Hz, CH_2O), 3.47 (d, 1H, *J* = 10.9 Hz, CH_2O), 5.12 (t, lH, *J* = 6.6 Hz, =CH-); ^{13}C NMR ($CDCl_3$) δ 17.7, 22.4, 23.2, 25.7, 38.4, 69.8 (CH_2O), 73.0 (-COH), 124.1 (=CH-), 132.1 $(CH_3)_2$ C=); Anal. Calcd. for $C_9H_{18}O_2$: C, 68.31; H, 11.46. Found : C, 68.24; H, 11.46.

(2*R*, 3*R*)-(-)-[3-$^2H_1$]-2,6-Dimethyl-5-heptadine-1,2-ol) ((+)-20 - d$_1$): Yield, 1.06 g (95 %) ; $[\alpha]_D^{28}$ -0.98º (c 1.00, $CHCl_3$); ^{1}H NMR δ 1.53 (t, 1H, *J* = 7.7 Hz, CHD), 2.06 (t, 2H, *J* = 7.7 Hz, CH_2CHD), 3.41 and 3.47 (AB dd, 2H, *J* = 11.0 Hz, CH_2O).

(2*S*, 3*S*)-(-)-[3-$^2H_1$]-2,6-Dimethyl-5-heptadine-1,2-ol) ((+)-20 - d_1) : Yield, 748 mg (94 %) ; $[\alpha]_D^{28}$ +0.94º (c 1.00, $CHCl_3$). (2*R*, 4*R*)-(-)-[4-$^2H_1$]-3,7-Dimethyl-1,6-octadine-3-ol ((3*R*, 4*R*)-(-)- [4-$^2H_1$]linalool, (-)-22- d_1[10,14] (-)-22- d_1: $[\alpha]_D^{26}$ -17.7º (C 1.00, $CHCl_3$) [lit $[\alpha]_D^{20}$ for the enantimer, +19.18º [15]]; IR (neat) ν_{max}, 3405 (OH), 2971 (CH), 2155 (w, CD), 1449 cm^{-1}; ^1H NMR δ 1.28 (s, 3H, CH_3), 1.53 (m, 1H, CHD), 1.57 (brs, lH, OH), 1.61 (s, 3H, =CCH_3), 1.68 (s, 3H, =CCH_3), 2.04 (brm, 2H, CH_2), 5.06 (dd, lH, *J* = 10.8, 1.3 Hz, *trans*-CH=CH_2), 5.11 (t, lH, *J* = 7.0 Hz, =CH-), 5.22 (dd, lH, *J* = 17.5, 1.3 Hz, *cis*-CH=CH_2), 5.92 (dd, lH, *J* = 17.5 Hz, -CH=CH_2); isotope ratio by field ionization MS analysis, 2.9 % d_0, 94.5 % d_1, 2.6 % d_2.

(2*S* 4*S*)-(+)-[4-$^2H_1$]-3,7-Dimethyl-1,6-octadine-3-ol ((3*S*, 4*S*)-(+)-[4-$^2H_1$]linalool (+)- 22-d_1) : Yield, 267 mg (43 %); $[\alpha]_D^{26}$ +17.7º (c 1.00, $CHCl_3$) isotope ratio by field ionization MS analysis, 2.9 % d_0, 96.2 % d_1, 0.9 % d_2.

(*E*)-(4*R*)-[4-$^2H_1$] -3.7-Dimehtyl-2,6-octadine-3-ol) ((4*R*)-4-$^2H_1$]geraniol, (4*R*)-23-d_1)[16]. A solution of 59 mg (0.38 mmol) of (-)-22- d_1 in 3 mL CH_2Cl_2 was added in one portion to a suspension of 328 mg (1.52 mmol) of pyridinium chlorochromate in 10 mL of CH_2Cl_2 and the reaction was followed by GLC and TLC. After stirring for 4.3 h at room temperature, the mixture was diluted with CH_2Cl_2 (10 mL) and filtered. The filtrate was washed with saturated aqueous $NaHCO_3$ (2 × 10 mL), saturated NaCl (10 mL) and water (10 mL). The organic fraction was dried ($MgSO_4$) and concentrated to obtain a crude mixture of (*E*)- and (*Z*)- aldehydes (*i.e.*, geranial and neral).

To a solution of the aldehyde mixture in 10 mL of ether was added 3 mL of 1 M $LiAlH_4$ in THF at 0 ºC. After stirring for 1 h at room temperature, the solution was quenched by adding 10 mL of saturated NH_4Cl at 0 ºC and slurry was filtered through Celite. The filter cake was washed with ether and separated aqueous layer from the filtrate was extracted with ether (2 × 20 mL). The combined organic fractions were dried ($MgSO_4$) and concentrated. Purification by flash chromatography with ethyl acetate:hexane (1:10, v/v) as eluent provided 24.5 mg (42 %) of (4*R*)-23- d_1 and 11.3 mg of (4*R*)-[4-$^2H_1$] nerol.

(4*R*)-23-d_1: IR (neat) ν_{max}, 3306 (OH), 2922 (CH), 2853 (CH) cm^{-1}: ^1H NMR δ 1.42 (s, lH, OH), 1.59 (s, 3H, CH_3), 1.66 (s, 6H, 2CH_3), 2.05 (m, 3H, CHD and CH_2), 4.14 (d, 2H, *J* = 7.0 Hz, CH_2O), 5.08 (brt, 1H, *J* ≅ 7 Hz, =CH-), 5.40 (t, lH, *J* ≅ 7 Hz, =CH-); isotope ratio by field ionization MS analysis, 1.2 % d_0, 98.1 % d_1, 0.0 % d_2.

(4*R*)- [4-$^2H_1$] nerol; IR (neat) ν_{max} 3414 (OH), 2924 (CH), 2855 (CH) cm^{-1}; ^1H NMR δ 1.31 (s, lH, OH), 1.59 (s, 3H, CH_3), 1.68 (s, 3H, CH_3), 1.73 (s, 3H, CH_3), 2.05 and 2.08 (m, 3H, CH_3 and CHD), 4.07 (d, 2H, *J* = 7.4 Hz, CH_2O), 5.08 (br, lH, =CH-), 5.42 (t, lH, *J* = 6.2 Hz, =CH-); isotope ratio by field ionization MS analysis, 0.5 % d_0, 97.5 % d_1, 1.8 % d_2. The enantiomeric purity of (-)-22- d_1 at C4 was -94 % due to the presence of 6 % of the (4*S*)-stereoisomer.

(*E*)-(4*S*)-[4-$^2H_1$]-3,7-Dimethyl-2,6-octadine-3-ol ((4*S*)-4-$^2H_1$]geraniol, (4*S*)-23-d_1 : Yield, 21.5 mg (34 %); IR (neat) ν_{max}, 3321 (OH), 2917 (CH), 2849 (CH) cm^{-1}; H NMR δ 1.36 (s, lH, OH), 1.60 (s, 3H, CH_3), 1.67 (s, 6H, 2CH_3), 2.08 (m, 3H, CHD and CH_2), 4.15 (t, 1H, *J* = 7.0 Hz, CH_2O), 5.08 (brt, lH, *J* ≅ 7 Hz, =CH-), 5.41 (t, lH, *J* ≅ 7 Hz, =CH-); isotope ratio by field ionization MS analysis, 2.5 % d_0, 97.5 % d_1.

(4*S*)- [4-$^2H_1$] nerol : Yield, 21.1 mg (32.9 %); IR (neat) ν_{max} 3340 (OH), 2963 (CH), 2924 (CH) cm^{-1}; ^1H NMR δ 1.33 (brs, lH, OH), 1.60 (s, 3H, CH_3), 1.68 (s, 3H, CH_3), 1.74 (s, 3H, CH_3), 2.06 and 2.08 (m, 3H, CH_2 and CHD), 4.08 (d, 2H, *J* = 7.4 Hz, CH_2O), 5.09 (br, lH, =CH-), 5.42 (t, lH, *J* = 6.7 Hz, =CH-1); isotope ratio by field ionization MS analysis, 1.7 % d_0, 98.3 % d_1. The enantiomeric purity of (+)-22- d_1 at C4 was -94 % due to contamination by 6 % of the (4*R*)-isomer.

RESULTS AND DISCUSSION

Pyrophosphorylation of (4*R*)- and (4*S*)-23- d_1 to (4*R*)- and (4*S*)-1- d_1 was performed according to a literature procedure in 25 % yield for each product[17]. The isotope content of both (4*R*)-and (4*S*)-I-d_1 were determined to be 2 % d_0 and 98.3 % d_1, based on GLC-MS anaylsis of olefins generated from these substrates by the cyclase preparations and the enantiomeric purity at C4 of both substrate was -94 %, based on the enantiomeric purities of the starting alcohols (+)- and (-)-22-d_1.

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