Multiple Rotamers of 3-(2,2,5,5-Tetramethyl-1-oxopyrrolinyl)-2-propen-1-ol, a Stereospecific Substrate of Liver Alcohol Dehydrogenase: Determination of Molecular Structure and Conformation by Electron Nuclear Double Resonance[1]

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Abstract: The molecular structure and conformation of the nitroxyl spin-label 3-(2,2,5,5-tetramethyl-1-oxypyrrolinyl)-2-propen-1-ol has been determined by electron nuclear double resonance (ENDOR) spectroscopy and molecular modeling. From ENDOR spectra of selectively deuterated analogues of the spin-labeled propenol in frozen solution, we have assigned resonance absorption features for each proton of the propenol side chain. From analysis of the dependence of the ENDOR spectra on H_0, we have identified the principal hyperfine coupling (hfc) components for each proton; from these couplings the dipolar hfc components were calculated in order to estimate electron–proton distances. Torsion angle search calculations were carried out to determine the conformational space compatible with both hard-sphere nonbonded constraints and ENDOR-determined electron–proton distance constraints. Molecular graphics analysis revealed that the double bond of the side chain is coplanar with the spin-label oxypyrrolinyl ring, exhibiting a single preferred structure, while the $-CH_2OH$ group exhibits multiple conformers due to rotation around the terminal C–C bond and the C–O bond. We also demonstrate that 3-(2,2,5,5-tetramethyl-1-oxypyrrolinyl)-2-(1-^{2}H)propenal is catalytically reduced by horse liver alcohol dehydrogenase (alcohol:NAD^+ oxidoreductase, EC 1.1.1.1.) to the spin-labeled propenol in a stereospecific manner.

Introduction

The chemically stable nitroxyl free-radical species known as spin-labels are widely used in biophysical studies as spectroscopic probes of macromolecular structure and dynamics.[2] Since the unpaired electron of nitroxyl spin-labels behaves as an effective point dipole, located at about the midpoint of the N–O bond of the nitroxyl group,[3] the hyperfine (hf[4]) interactions of nearby nuclei with the unpaired electron are largely dipole–dipole in character. We have shown that this hf coupling can be accurately measured by electron nuclear double resonance (ENDOR) spectroscopy and that ENDOR of nitroxyl spin-labels in combination with molecular modeling provides a general method for determination of structure and conformation of molecules in solution through measurements of electron–nucleus distances.[5] The accuracy of these distance measurements in the range of 4–11 Å is exceeded only by that of single-crystal X-ray diffraction methods.

In the accompanying publication we demonstrate on the basis of ENDOR and molecular modeling studies that oxypyrrolinyl spin-labels with polyene side chains containing a terminal carbonyl group exhibit an all planar *trans* structure.[6] In this investigation we report the ENDOR-determined structure of an oxypyrrolinyl spin-label with an olefinic alcoholic side chain that exhibits three distinct conformations of the terminal $-CH_2OH$ group while the two olefinic groups, one in the propenol side chain and the other in the oxypyrroline ring, are *trans* to each other. In the accompanying publication the single preferred conformation of the side chain is explained mainly in terms of extended π-conjugation and resonance. However, in the propenol side chain, the terminal carbon atom is not sp^2 hybridized, and, therefore, rotation around the terminal C(sp^2)–C(sp^3) bond is not restricted. This study provides direct experimental evidence of multiple conformers of the $-CH_2OH$ group. We also provide a detailed description of the three-dimensional structures of the rotamers of the terminal alcoholic group.

Experimental Procedures

Materials. Organic solvents and reagents were employed as described in the preceding publication.[6] Crystalline (horse) liver alcohol dehydrogenase (LADH,[4] alcohol:NAD^+ oxidoreductase, EC 1.1.1.1) and NADH were obtained from Boehringer Mannheim Biochemicals (Indianapolis, IN 46250). Deuterated solvents were obtained from Cambridge Isotope Laboratories, Inc. (Woburn, MA 01801). LADH stock solutions were prepared by dissolving 100 mg of the crystalline enzyme overnight in 1 mL of 1 M KCl and 5×10^{-3} M sodium cacodylate at pH 7.0 buffer, removing insoluble material by centrifugation, and then dialyzing the enzyme solution twice against 500 mL of 0.2 M KCl,

(1) This work was supported by grants from the National Institutes of Health (AA 06374 and MH 47809).

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(4) The following abbreviations are used: EPR, electron paramagnetic resonance; ENDOR, electron nuclear double resonance; hf, hyperfine; hfc, hyperfine coupling; LADH (horse) liver alcohol dehydrogenase; NAD^+, oxidized nicotinamide adenine dinucleotide; NADH, reduced nicotinamide adenine dinucleotide; PIPES, 1,4-piperazinediethanesulfonic acid; rf, radiofrequency; THF, tetrahydrofuran.

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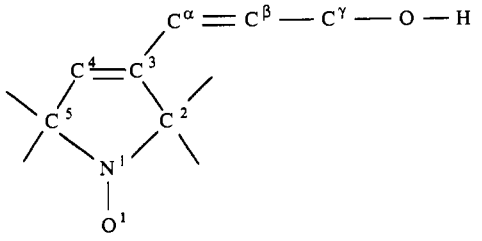

I. SL - $C^{\alpha}H = C^{\beta}H - C^{\gamma}H_2 - OH$

II. SL - $C^{\alpha}D = C^{\beta}H - C^{\gamma}H_2 - OH$

III. SL - $C^{\alpha}H = C^{\beta}H - C^{\gamma}HD - OH$

IV. SL - $C^{\alpha}H = C^{\beta}H - C^{\gamma}D_2 - OH$

Figure 1. Illustration of the atomic numbering scheme of 3-(2,2,5,5-tetramethyl-1-oxypyrrolinyl)-2-propen-1-ol and the specifically deuterated analogues used in this study. SL refers to the spin-label 3-(2,2,5,5-tetramethyl-1-oxypyrrolinyl) moiety. In the atomic numbering scheme for the side chain, we designate atom positions using Greek letters, beginning with the carbon atom attached to the spin-label moiety. The positions of deuterium substitutions are indicated by D rather than ^2H.

containing 5×10^{-3} M cacodylate at pH 7.0 (all steps done at 4 °C). Protein concentration was determined by the pyrazole titration method.[7]

In Figure 1 are illustrated the structural formulae of the spin-labeled propen-1-ol and its deuterated analogues **I–IV** synthesized and characterized in this investigation. The syntheses of these compounds are outlined below.

3-(2,2,5,5-Tetramethyl-1-oxypyrrolinyl)-2-propen-1-ol (I). The acid chloride of 3-(2,2,5,5-tetramethyl-1-oxypyrrolinyl)-2-propenoic acid was synthesized by reaction of 1.18 g (5.6 mmol) of the acid with $SOCl_2$, as described in the preceding paper.[6] The oily product was dissolved in 20 mL of THF and placed under dry nitrogen gas, and a solution of LiAlH-(O-*t*Bu)$_3$ (5.71 g, 22.5 mmol) in 35 mL of THF was added dropwise over 60 min with vigorous stirring at 0 °C. The mixture was stirred 1 h at room temperature and then poured over approximately 600 mL of crushed ice. The melted aqueous mixture was suction filtered, and the filtrate was extracted with ethyl acetate until the organic extracts were colorless. The combined organic extracts were washed with 5 mL of 5% $NaHCO_3$ and 5 mL of water, dried over $MgSO_4$, and evaporated to an oil. This was dispersed in about 200 mL of pentane, and the pentane was evaporated in vacuo to produce a crystalline product. The pure product was obtained by recrystallization from ether at –20 °C (mp 75.3–75.9 °C), yielding 0.68 g (61%). Anal. Calcd for $C_{11}H_{18}NO_2$ (Found): C, 67.32 (67.50); H, 9.24 (9.12); N, 7.14 (7.10). Mass spectra of **I** showed the highest molecular ion peak to correspond to the expected *m/e* ratio of 196 with the characteristic breakdown pattern of an oxypyrrolinyl spin-label species.[8]

3-(2,2,5,5-Tetramethyl-1-oxopyrrolinyl)-2-(3-^{2}H)propen-1-ol (**II**) was synthesized from 0.4 g (1.9 mmol) of spin-labeled 3-(2,2,5,5-tetramethyl-1-oxypyrrolinyl)-2-(3-^{2}H)propenoic acid[6] as the starting material according to the procedure for **I** to yield 0.2 g (55% yield) of the deuterated alcohol (mp 75.3–75.9 °C). Anal. Calcd for $C_{11}H_{17}NO_2D$ (Found): C, 66.97 (66.94); H, 9.70 (9.58); N, 7.10 (7.10). Mass spectra of **II** showed the highest molecular ion peak to correspond to the expected *m/e* ratio of 197, and no evidence of the presence of **I** was observed in the mass spectrum of **II**.

3-(2,2,5,5-Tetramethyl-1-oxypyrrolinyl)-2-(1-^{2}H)propen-1-ol (**III**) was synthesized by stereoselective enzymatic reduction[9] of the deuteroaldehyde 3-(2,2,5,5-tetramethyl-1-oxypyrrolinyl)-2-(1-^{2}H)propenal,[6] using LADH and the coenzyme NADH. The deuteroaldehyde (0.1 g, 0.51 mmol) and NADH (1.0 g, 1.4 mmol) were dissolved in a 40 mL solution of 0.1 M KCl buffered to pH 7 with 0.1 M PIPES. A 50-μL aliquot of LADH (1×10^{-3} N in active sites) was added to the solution, and the mixture was stirred gently at room temperature. At 5 min intervals a 5-μL aliquot was removed from the reaction mixture and diluted into 3 mL of water, and the absorbance at 340 nm of NADH in the diluted sample was measured. The value of A_{340} in the sample dropped steadily until 30 min had passed, after which no further change in A_{340} was observed. After 40 min the reaction was considered to have reached completion. The alcohol product was then extracted into ethyl acetate and purified as described above in the procedure for **I**. The yield was 0.08 g (80% yield) of the pure product (mp 75–76 °C). Mass spectra of **III** showed the highest molecular ion peak to correspond to the expected *m/e* ratio of 197.

To synthesize 3-(2,2,5,5-tetramethyl-1-oxypyrrolinyl)-2-(1,1-$^2H_2$)-propen-1-ol (**IV**), the spin-labeled 2-propenoic acid (1.0 g, 4.8 mmol) was converted to its acid chloride and reduced with LiAl^{2}H(O-*t*Bu)$_3$ (4.88 g, 19.2 mmol) to yield 0.43 g (46%) of the pure product (mp 75.3–75.9 °C). Anal. Calcd for $C_{11}H_{16}NO_2D_2$ (Found): C, 66.64 (66.69); H, 10.16 (9.95); N, 7.06 (7.09). Mass spectra of **IV** showed the highest molecular ion peak to correspond to the expected *m/e* ratio of 198.

Enzyme Kinetics. Initial velocity data were collected spectrophotometrically to determine the steady-state kinetic parameters k_{cat} and k_{cat}/K_M for the oxidation of **I** and the reduction of 3-(2,2,5,5-tetramethyl-1-oxypyrrolinyl)-2-propenal[6] (**V**[10]) catalyzed by LADH, where K_M is the Michaelis constant for the second substrate (**I** or **V**) under conditions of saturating concentrations of NAD^+ or NADH, respectively, as the leading substrate. The absorbance change at 340 nm due to oxidation of NADH or the reduction of NAD^+ was followed with a Cary 15 recording spectrophotometer modified by On-Line Instrument Systems, Inc. (Jefferson, GA 30549) for microprocessor-controlled data acquisition. A vibrating reed stirring assembly previously described from this laboratory[11] was used for collection of kinetic data. In general, initial velocity data were evaluated with use of the algorithm ENZKIN provided by Professor J. Westley of the Department of Biochemistry and Molecular Biology at The University of Chicago, as previously described.[12]

EPR and ENDOR. Spectroscopic studies were carried out as described in the preceding paper.[6] Spin-labeled compounds were dissolved to a concentration of 5×10^{-3} M in ($^2H_4$)methanol or (^{2}H)chloroform:($^2H_8$)-toluene:($^2H_6$)dimethyl sulfoxide (25:25:50 by volume) for EPR and ENDOR spectroscopy. ENDOR spectra were collected with ~8-kHz modulation depth of the rf field and at a sample temperature of ~20 K, as described previously.[13] For collection of ENDOR spectra, the settings A and B of the static laboratory magnetic field refer to the low-field and central-field EPR absorption features of the nitroxyl spin-labels, as defined in the preceding publication.[6] The interpretation of ENDOR spectra of spin-labeled molecules in terms of principal hfc components and calculated dipolar electron–proton distances has been described previously.[5,6]

Molecular Modeling. The molecular model of spin-labeled propenol was constructed from X-ray-defined molecular fragments. Coordinates of the non-hydrogen atoms of the 2,2,5,5-tetramethyl-1-oxypyrrolinyl moiety were taken from the X-ray-determined structure of 2,2,5,5-tetramethyl-1-oxypyrroline-3-carboxamide.[14] The side chain of **I** was constructed on the basis of bond lengths and bond angles of non-hydrogen atoms of similar compounds determined by X-ray diffraction studies,[14,15] as described in the preceding paper.[6] For the terminal –CH_2OH group, we have assumed the C–H and C–O bond lengths and valence angles of an idealized methanol molecule.[16] The terminal C(sp^2)–C(sp^3) bond length was set to 1.501 Å. Molecular modeling and torsion angle search calculations were carried out with the programs SYBYL[17] and FRODO,[18]

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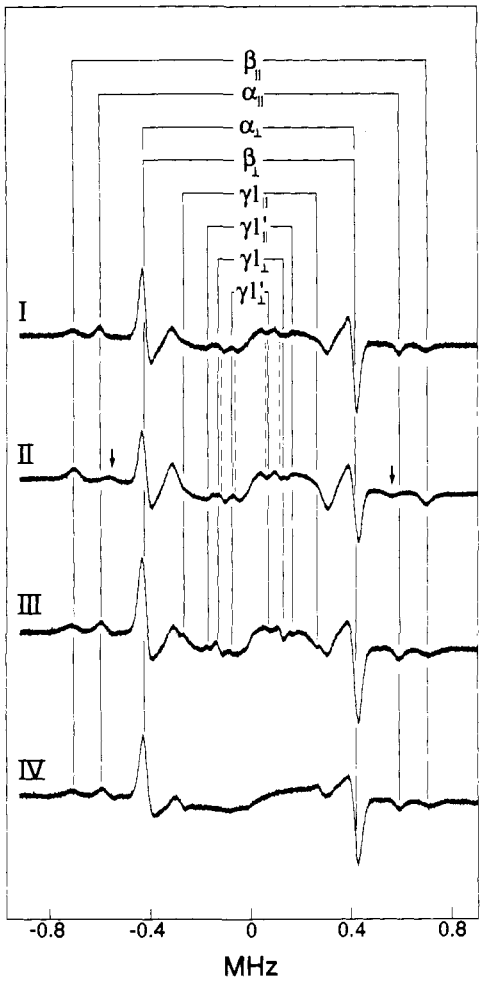

Figure 2. Proton ENDOR spectra of **I**–**IV** in ($^2H_4$)methanol with H_0 at setting B.[6] The ENDOR splittings for H^{α}, H^{β}, and $H^{\gamma 1}$ are identified in the stick diagram (solid lines) and are labeled according to their principal hfc components. Line pairs indicated by dashed lines are from $H^{\gamma 2}$. These splittings are different from those of $H^{\gamma 1}$. Two weak features indicated by arrows are assigned to the parallel hfc component of the vinylic proton of the oxypyrrolinyl ring (see ref 5f). An additional pair of resonances is observed near the features assigned to the perpendicular hfc components of H^{α} and H^{β}, and these two resonances overlap with the parallel hfc components of the γ protons. These features are also observed in the spectrum of **IV**. Since the ENDOR features of H^{α} and H^{β} are clearly identified on the basis of selective deuteration, we ascribe these extra features to impurities.

as previously described.[5,6] In Figure 1, the atomic numbering scheme is shown for purposes of discussing molecular modeling results.

Results and Discussion

A. Steady-State Kinetic Studies of Spin-Label Substrate Turnover. In contrast to yeast alcohol dehydrogenase, LADH readily catalyzes the oxidation or reduction of a variety of bulky aromatic alcohols and aldehydes,[19] the most pertinent for purposes of this investigation perhaps being *N,N'*-dimethylcinnamaldehyde.[20] To establish the specificity of reactivity of substrates **I** and **V**,[10] we have carried out steady-state kinetic studies of their LADH-catalyzed oxidation and reduction, respectively, to determine kinetic parameters. These studies followed the methods used earlier to characterize the enzyme-catalyzed oxidation of benzyl alcohol.[12] With saturating concentrations of NAD^+ as the leading substrate in 0.1 M KCl, buffered to pH 8 with 0.03 M PIPES, initial velocity data for the oxidation of **I** yielded values of $\sim 14.4 \pm 0.5$ s^{-1} for k_{cat} and $(3.79 \pm 0.26) \times 10^{-3}$ M for K_M. Under saturating concentrations of NADH as the leading substrate, initial velocity data for the LADH-catalyzed reduction of **V** at pH 7 yielded values of 32.1 ± 1.2 s^{-1} for k_{cat} and $(1.48 \pm 0.13) \times 10^{-3}$ M for K_M. These results show that the spin-labels **I** and **V** are as catalytically reactive and kinetically specific as other classical substrates employed hitherto for LADH.[12,19]

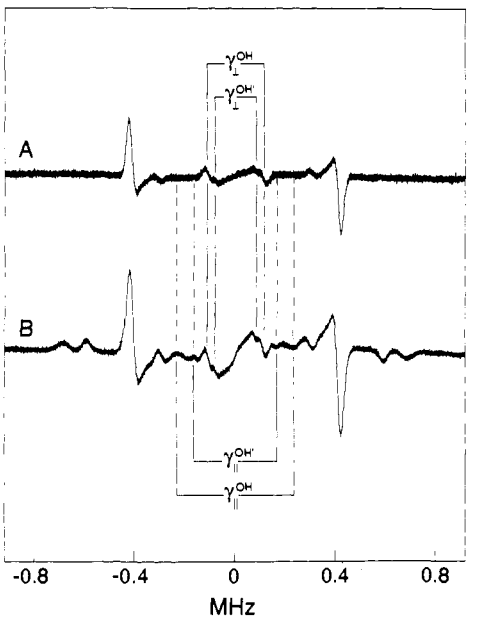

Figure 3. Proton ENDOR spectra of compound **IV** in ($^2H_6$)DMSO:(^2H)chloroform:($^2H_8$)toluene with H_0 at settings A (top) and at B (bottom). In the stick diagram the ENDOR splittings for the hydroxyl protons are identified. Two sets of hfc components are identified for H^{OH} and are labeled γ^{OH} and $\gamma^{OH'}$. The ENDOR features for the α and β protons are not labeled (see Figure 2). Other conditions are as in Figure 2.

B. Assignment of ENDOR Resonances and Estimation of Electron–Proton Distances in Spin-Labeled Propenol. In Figure 2 are illustrated the proton ENDOR spectra of **I**–**IV** dissolved in perdeuterated methanol with H_0 at setting B. At this H_0 setting, both the parallel and the perpendicular hfc components are observed in the ENDOR spectrum. The resonance features of H^{α} and H^{β} are identified by comparing the spectra of **I** and **II**. Two line pairs are seen for each of these two protons, and they are assigned to the parallel and perpendicular hfc components. On the other hand, resonance features for the two γ protons are not easily identifiable, as seen in the spectra of **I** and **II** in Figure 2. Although the hydroxyl proton, which exchanges with solvent deuterons, contributes no resonance feature to these spectra, the resonance absorption features for the two γ protons severely overlap. However, the two sets of resonance features were resolved by selective deuteration at the γ position, creating the enantiomorphically pure[9] RCHDOH analogue **III**. This was accomplished by stereospecific enzyme-catalyzed reduction of the deuteroaldehyde with use of NADH. The ENDOR spectrum of **III** in Figure 2 reveals two well-resolved perpendicular hfc components, labeled $\gamma 1_{\perp}$ and $\gamma 1_{\perp}'$, and their corresponding parallel hfc components. The presence of two sets of parallel and perpendicular hfc components for the γ proton indicates the existence of at least two conformers differing in their electron–proton separations. The peak-to-peak amplitudes for the second set of resonance features, labeled $\gamma 1_{\parallel}'$ and $\gamma 1_{\perp}'$, are much smaller than those of the first set, indicating that the relative population of the second conformer is smaller than that of the first.

The stereospecificity of this enzymatic reduction is confirmed by the ENDOR spectrum of the resulting alcohol **III**, which

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Table I. Summary of hfc Components and Estimated Electron–Proton Distances in 3-(2,2,5,5-Tetramethyl-1-oxypyrrolinyl)-2-propen-1-ol

proton[a]	hfc component (MHz) $A_{\parallel}$	$A_{\perp}$	A_{iso}	$A_{\parallel}^{D}$	$A_{\perp}^{D}$	r^{b} (Å)
α	1.199	0.836	−0.158	1.357	−0.678	4.90 ± 0.02
β	1.431	0.836	−0.080	1.511	−0.756	4.71 ± 0.02
$\gamma 1$	0.512	0.256	0.000	0.512	−0.256	6.76 ± 0.06[c]
$\gamma 1'$	0.340	0.156	0.009	0.331	−0.165	7.82 ± 0.10[c]
OH	0.469	0.236	−0.001	0.470	−0.235	6.96 ± 0.06[d]
OH′	0.331	0.167	−0.001	0.332	−0.166	7.82 ± 0.08[d]

[a] ENDOR line pairs for each proton are assigned from ENDOR spectra of compounds I–IV in Figures 2 and 3. [b] The uncertainty in the frequency of 0.010–0.016 MHz due to the line width of each absorption is included in the calculation of electron–proton distances. [c] These two sets of resonance features, labeled $\gamma 1$ and $\gamma 1'$, come from one γ proton and are assigned unambiguously to two conformations. A clear assignment of resonance features of the second γ proton is not possible, and therefore they are not included in this table (see text). [d] The two sets of resonance features for the hydroxyl proton, labeled, γ^{OH} and $\gamma^{OH'}$, are assigned unambiguously to two conformations.

showed that one set of resonances for γ protons had completely vanished, while the other was undiminished in intensity compared to that for the fully protonated compound. If the enzymatic reduction proceeded without stereospecificity, 50% of the $\gamma 1$ proton and 50% of the $\gamma 2$ proton position would have been deuterated. Under these circumstances, both sets of resonances would have appeared in the ENDOR spectra but with decreased intensity. By comparing the ENDOR spectrum of **III** with that of **I** and **II**, two additional resonance features can be identified for the second γ proton, indicated by dashed lines in Figure 2. However, the ENDOR features for this second γ proton are so closely spaced that unambiguous assignment of its hfc components is not feasible from the spectra of **I** and **II**.

Figure 3 illustrates the ENDOR spectra of **IV** in the ternary perdeuterated dimethyl sulfoxide:chloroform:toluene solvent mixture. Since the ENDOR features for H^{α} and H^{β} and their line splittings are identical to those seen for **IV** in Figure 2, they are not labeled. Use of an aprotic solvent system provides an opportunity to detect the resonance features of the hydroxyl proton that normally is rapidly exchanged with deuterons in ($^2H_4$)-methanol. The ENDOR spectra of **IV** in Figure 3 show at least two sets of hfc components for the hydroxyl proton, labeled γ^{OH} and $\gamma^{OH'}$. These results indicate that for spin-labeled propenol there are at least two distinct rotamers of the hydroxyl group.

In Table I we have summarized the observed hfc components for each proton of **I**. We have also listed the isotropic and the dipolar hfc components and corresponding electron–proton separations for each proton. Since the principal hfc components for the second γ proton cannot be unambiguously assigned, their values and the corresponding electron–proton distance are not listed in Table I.

C. Conformation of Spin-Labeled Propenol. To determine the conformations of spin-labeled propenol, we have carried out computer-based torsion angle search calculations for all bonds in the side chain constrained within van der Waals hard-sphere limits to the ENDOR-determined electron–proton separations in Table I. The general methodology described in the preceding publication[6] was applied.

The ENDOR data indicate that spin-labeled propenol exhibits at least two conformers. Since two sets of electron–proton distances were obtained for $H^{\gamma 1}$ and H^{OH} ($r^{\gamma 1}$, $r^{\gamma 1'}$ and r^{OH}, $r^{OH'}$), as listed in Table I, we have carried out torsion angle search calculations for each case separately. Four separate calculations were performed with the following distance constraints applied simultaneously: (1) $r^{\gamma 1}$ and r^{OH}; (2) $r^{\gamma 1}$ and $r^{OH'}$; (3) $r^{\gamma 1'}$ and r^{OH}; and (4) $r^{\gamma 1'}$ and $r^{OH'}$. In each of these calculations the electron–proton distance constraints to H^{α} and H^{β} listed in Table I were also applied. We discuss the results for rotation about each bond separately.

The ENDOR-determined distance constraints to H^{α} and H^{β} in spin-labeled propenol resulted in an essentially planar *trans* structure of the $C^{\alpha}{=}C^{\beta}$ group with respect to the olefinic group in the oxypyrrolinyl ring. The two distance constraints to $H^{\gamma 1}$ gave two distinct families of conformations around the C^{β}–C^{γ} bond. Finally, the two distance constraints to H^{OH} were used to calculate conformations around the C^{γ}–O bond. No conformation was found which simultaneously fit the distance constraints $r^{\gamma 1'}$ and $r^{OH'}$. However, simultaneous application of the distance constraints $r^{\gamma 1}$ and r^{OH}, $r^{\gamma 1}$ and $r^{OH'}$, and $r^{\gamma 1'}$ and r^{OH} resulted in three distinct families of conformations around the C^{γ}–O bond. In Table II we have listed the dihedral angles for the three conformations that were compatible with both van der Waals constraints and the ENDOR-determined distance constraints in Table I.

Table II. Values of Dihedral Angles[a] of ENDOR-Constrained Conformations of 3-(2,2,5,5-Tetramethyl-1-oxypyrrolinyl)-2-propen-1-ol

dihedral angle	τ (deg) conformer A	conformer B	conformer C
[C(4)=C(3)—$C^{\alpha}{=}C^{\beta}$]	180 ± 10	180 ± 10	180 ± 10
[C(3)—$C^{\alpha}{=}C^{\beta}$—C^{γ})	170 ± 6	170 ± 6	170 ± 6
[$C^{\alpha}{=}C^{\beta}$—C^{γ}—O]	−115 ± 12	−115 ± 12	−20 ± 20
[C^{β}—C^{γ}—O—H]	−60 ± 12	160 ± 20	78 ± 18
[H^{α}—$C^{\alpha}{=}C^{\beta}$—H^{β}]	170 ± 6	170 ± 6	170 ± 6
[H^{β}—C^{β}—C^{γ}—$H^{\gamma 1}$]	−175 ± 12	−175 ± 12	−80 ± 20
[$H^{\gamma 1}$—C^{γ}—O—H]	180 ± 12	40 ± 12	−42 ± 18

[a] Dihedral angles are designated according to the atomic numbering scheme in Figure 1.

As seen in Table II, conformers A and B differ only in the dihedral angle around the C^{γ}–O bond. The conformers identified for the terminal $-CH_2OH$ group are illustrated in the form of Newman diagrams in Figure 4. The top diagram illustrates the two ENDOR-constrained rotamers around the C^{β}–C^{γ} bond calculated using $r^{\gamma 1}$ distance constraint in one case and $r^{\gamma 1'}$ in the other. As seen in the top diagram of Figure 4, one conformer has a nearly eclipsed conformation, while the other has a staggered conformation, as measured by the [$C^{\alpha}{=}C^{\beta}$—C^{γ}—O] dihedral angle. The eclipsed structure, which is compatible with $r^{\gamma 1'}$, would be less favorable energetically and is expected to be of lower population, and indeed the $\gamma 1'$ resonance features are much weaker than the $\gamma 1$ features, as seen in the ENDOR spectrum of **III** in Figure 2. These results suggest that of the two rotamers around the C^{β}–C^{γ} bond the predominant conformer is fully staggered and that the second (eclipsed) conformer exists only in low population.

The bottom diagram in Figure 4 illustrates the ENDOR-constrained rotamers around the C^{γ}–O bond. The values of the [C^{β}–C^{γ}–O–H] dihedral angle for these three families of conformations are listed in Table II as conformers A, B, and C. The conformational space indicated by dots is obtained by simultaneous application of the distance constraints $r^{\gamma 1'}$ and r^{OH}. As discussed above, this conformation (conformer C) should be of low population because it is associated with the $\gamma 1'$ features. The other two families are compatible with the $r^{\gamma 1}$ constraint together with the r^{OH} constraint in one case and with the $r^{OH'}$ constraint in the other case. In these two conformations the hydroxyl proton is either *gauche* or *trans* with respect to the C^{β} atom.

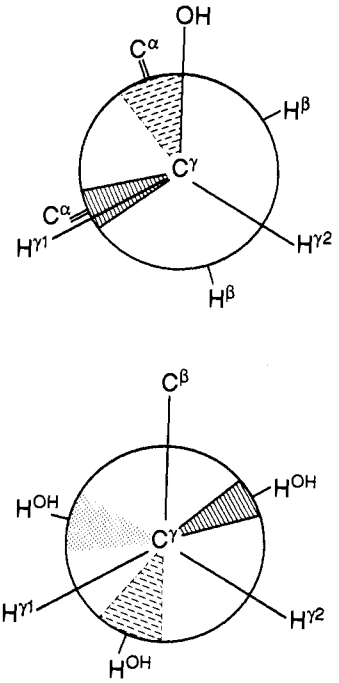

Figure 4. Newman diagrams illustrating the range of ENDOR-constrained dihedral angles [C^α=C^β—C^γ—O] and [C^β—C^γ—O—H] in spin-labeled propenol. The top diagram corresponds to a projection down the C^γ—C^β bond, and the bottom diagram illustrates the projection down the C^γ—O bond. In the top diagram the area indicated by solid lines corresponds to the range of [C^α=C^β—C^γ—O] angles compatible with the ENDOR-determined distance constraint $r^{\gamma 1}$, and the area indicated by dashed lines is obtained from $r^{\gamma 1'}$. The average positions of C^α and H^β for these two conformers are also indicated in the diagram. In the bottom diagram three regions of conformational space are illustrated. The area with the solid-line hatch marks is obtained using $r^{\gamma 1}$ and r^{OH} as constraints (conformer **A**), while the area with the dashed lines is obtained using $r^{\gamma 1}$ and $r^{OH'}$ (conformer **B**). The area indicated by dots is obtained by application of the distance constraints $r^{\gamma 1'}$ and r^{OH} (conformer **C**). See Table II.

Furthermore, the [$H^{\gamma 1}$–C^γ–O–H] dihedral angles of 180 ± 12° and 42 ± 12° for these conformers are close to those expected for ideal stereoisomers.

D. Comparison of the ENDOR-Determined Structure of Spin-Labeled Propenol with the Structures of Allyl Alcohols. By applying ENDOR, we have previously shown that it is feasible to identify multiple conformers of molecules in solution.[5c,d] In the accompanying publication on spin-labeled alkene aldehydes, acids, and esters, the single preferred conformation of the side chain of each of these compounds as determined by ENDOR is explained primarily in terms of extended π-conjugation and resonance.[6] In this investigation, however, the ENDOR spectra of spin-labeled propen-1-ol indicate the existence of multiple conformers of the $-CH_2OH$ group. This is not surprising since the terminal carbon atom is not sp^2 hybridized and rotation around the terminal C–C bond of the side chain is not restricted as it is in conjugated systems. The ENDOR-determined distance constraints to the side chain protons in spin-labeled propenol resulted in: (1) an essentially planar *trans* structure of the C^α=C^β group with respect to the olefinic group in the oxypyrrolinyl ring; (2) two distinct families of conformers around the C^β–C^γ bond; and (3) three orientations of the OH group with respect to the rest of the molecule.

For the allyl alcohol side chain in spin-labeled 2-propen-1-ol, the rotamers can be characterized by two dihedral angles [C^α=C^β—C^γ—O] and [C^β—C^γ—O—H]. Conformations of molecules are generally described as synperiplanar (*sp*), synclinal (*sc*), anticlinal (*ac*), or antiperiplanar (*ap*) according to whether the dihedral angle is within ±30° of 0°, ±60°, ±120°, or ±180°, respectively.[21] As seen in Figure 4, the two families of conformers around the C^β–C^γ bond differ by 120° in the value of the [C^α=C^β—C^γ—O] dihedral angle. In one case the hydroxyl oxygen atom is anticlinal with respect to the C^α=C^β bond, and in the second case the oxygen atom is synperiplanar with respect to the olefinic group. Also, as seen in Figure 4, the hydroxyl hydrogen atom is either synclinal or nearly antiperiplanar relative to the C^β atom. Therefore, the three ENDOR-determined rotamers of the allyl alcohol side chain in spin-labeled 2-propen-1-ol, listed as conformers A, B, and C in Table II, can be described as (*ac*, *sc*), (*ac*, *ap*), and (*sp*, *sc*), respectively.

Thus far, the molecular structures of allyl alcohols have been elucidated primarily on the basis of microwave spectroscopy and electron diffraction studies of molecules in the gas phase.[22] In these studies, the rotamer mixture made the analysis too complex to obtain reliable estimates of all independent structural parameters. However, these studies demonstrate the existence of more than one conformation of the $-CH_2OH$ group in allyl alcohols and led to the conclusion that in the gaseous state the (*ac*, *sc*) and (*sp*, *sc*) forms of molecules exist in almost equal population.[22] Microwave studies of allyl alcohols at low temperatures (approximately –70 °C) indicate that the (*ac*, *sc*) form is the most stable.[22a] The ENDOR study presented here for spin-labeled propenol demonstrates that the predominant conformer around the C^β–C^γ bond is anticlinal, while the rotamers around the C^γ–O bond can be either synclinal or antiperiplanar.

(21) *IUPAC-Nomenclature of Organic Chemistry, Section E: Stereochemistry (Recommendations 1974)*; Pergamon Press: New York, 1979; pp 473–490.

(22) (a) Murty, A. N.; Curl, R. F., Jr. *J. Chem. Phys.* **1967**, *46*, 4167–4180. (b) Caminati, W.; Velino, B.; Fantoni, A. C. *Mol. Phys.* **1987**, *61*, 1269–1282. (c) Vanhouteghem, F.; Pyckhout, W.; van Alsenoy, C.; van den Enden, L.; Geise, H. J. *J. Mol. Struct.* **1986**, *140*, 33–48.