

# Synthesis of Conjugated Polyene Carbonyl Derivatives of Nitroxyl Spin-Labels and Determination of Their Molecular Structure and Conformation by Electron Nuclear Double Resonance<sup>1</sup>

Devkumar Mustafi, William E. Boisvert, and Marvin W. Makinen\*

Contribution from the Department of Biochemistry and Molecular Biology, Cummings Life Science Center, 920 E. 58th Street, The University of Chicago, Chicago, Illinois 60637

Received September 1, 1992

**Abstract:** Formic, propenoic, and pentadienoic acid derivatives of the nitroxyl spin-label 2,2,5,5-tetramethyl-1-oxypyrroline and their corresponding ethyl ester and aldehyde analogues have been synthesized and characterized by chemical methods. Because the  $\pi$ -bonding orbitals of the olefinic side chains are conjugated to the vinyl group of the oxypyrroline ring, these spin-labeled derivatives are excellent spectrophotometric probes, exhibiting high molecular absorption extinction in the near ultraviolet. The molecular structures and conformations of these spin-labeled derivatives have been determined by electron nuclear double resonance (ENDOR) spectroscopy and molecular modeling. ENDOR was performed on frozen, glassy solutions of the spin-labels, and ENDOR absorptions of protons on the side chains were assigned by selective deuteration. From the maximum and minimum ENDOR shifts that correspond to the principal hyperfine coupling components, the dipolar hyperfine contributions were calculated to estimate electron–proton separations. The electron–proton distances evaluated over a 4.5–10.5-Å range were associated with less than 3% uncertainty on the basis of ENDOR line widths. Conformational analysis based on torsion angle search calculations, constrained by ENDOR-determined electron–proton separations, showed that the polyene chain has an all planar, *trans* conformation. In the aldehydes the conformation of the C=O group with respect to the alkene chain is found to be *s-trans*, while in the acids and esters the -OH and -OC<sub>2</sub>H<sub>5</sub> groups were found to be *s-trans*, placing the C=O group in an *s-cis* conformation. The different conformations of the carbonyl group in these compounds are explained on the basis of resonance energy and dipolar interactions of the polyene side chain and the carbonyl or carboxyl group.

## Introduction

Insight into the stereochemistry of unsaturated aldehydes, acids, and esters is germane to understanding many important problems in chemistry and biochemistry, one interesting example being the *cis*–*trans* isomerization of the carotenoid retinal.<sup>2</sup> The  $\pi$ -electron conjugation in these molecules, in general, results in one of two conformations, planar *cis* or *trans*. There have been few successful investigations of the precise molecular structures and the preferred conformations of these molecules in solution. Conformational analyses of unsaturated carbonyl derivatives have been carried out on the basis of dipolar moment measurements,<sup>3</sup> infrared and microwave spectroscopy,<sup>4</sup> nuclear magnetic resonance<sup>5</sup> (NMR<sup>6</sup>), and potential energy calculations.<sup>7</sup> These techniques are generally not sufficiently sensitive to assign precise molecular structures in solution, particularly with respect to the conformation of the

terminal functional group of aldehydes, acids, or esters, and there are no decisive criteria to differentiate *cis* and *trans* conformers since the resonances of protons on opposite sides of an olefinic bond are extremely difficult to resolve in NMR experiments.

Here we investigate conformational properties of conjugated polyene aldehydes, acids, and esters by electron nuclear double resonance (ENDOR<sup>6</sup>) spectroscopy. We have synthesized the ethyl ester, carboxylic acid, and aldehyde forms of formyl, propenoyl, and pentadienoyl derivatives of the nitroxyl spin-label 2,2,5,5-tetramethyl-1-oxypyrroline. We have shown that ENDOR spectroscopy of nitroxyl spin-labels provides a general method for determining the structure of molecules in frozen solution through measurement of electron–nucleus distances. The distances obtained in the range of 4–11 Å are determined with an accuracy that is exceeded only by single-crystal X-ray diffraction methods.<sup>8</sup> We apply this methodology to structure determination of the conjugated olefinic spin-labeled compounds described here. The ENDOR shifts of nuclei for molecules in frozen solutions are determined both by through-bond (Fermi) interactions and by through-space (dipolar) interactions. The dipolar hfc components obtained by ENDOR yield direct estimates of electron–nucleus distances. Since the unpaired electron spin is almost entirely localized to the nitroxyl nitrogen and oxygen<sup>9</sup> and can be considered as an effective point dipole located 0.57 Å from the nitrogen along the N–O bond,<sup>10</sup> the electron–nuclear dipolar separations yield nuclear coordinates

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

(2) Wald, G. *Science* **1968**, *162*, 230–239.

(3) (a) Estok, G. K.; Dehn, J. S. *J. Am. Chem. Soc.* **1955**, *77*, 4769–4770.

(b) Hannay, N. B.; Smyth, C. P. *J. Am. Chem. Soc.* **1946**, *68*, 1357–1360. (c) Rogers, M. T. *J. Am. Chem. Soc.* **1947**, *69*, 1243–1246. (d) Hurdis, E. C.; Smyth, C. P. *J. Am. Chem. Soc.* **1943**, *65*, 89–96.

(4) (a) Turner, R. B.; Voitle, D. M. *J. Am. Chem. Soc.* **1951**, *73*, 1403–1410. (b) Cherniak, E. A.; Costain, C. C. *J. Chem. Phys.* **1966**, *45*, 104–110. (c) Curl, R. F., Jr. *J. Chem. Phys.* **1959**, *30*, 1529–1536.

(5) (a) Rowan, R., III; McCammon, J. A.; Sykes, B. D. *J. Am. Chem. Soc.* **1974**, *96*, 4773–4780. (b) Honig, B.; Hudson, B.; Sykes, B. D.; Karplus, M. *Proc. Natl. Acad. Sci. U.S.A.* **1971**, *68*, 1289–1293. (c) Rowan, R., III; Warshel, A.; Sykes, B. D.; Karplus, M. *Biochemistry* **1974**, *13*, 970–981.

(6) The following abbreviations are used: EPR, electron paramagnetic resonance; ENDOR, electron nuclear double resonance; hf, hyperfine; hfc, hyperfine coupling; NMR, nuclear magnetic resonance; rf, radio frequency; THF, tetrahydrofuran.

(7) (a) Liljefors, T.; Allinger, N. L. *J. Am. Chem. Soc.* **1976**, *98*, 2745–2749. (b) Allinger, N. L.; Tai, J. C. *J. Am. Chem. Soc.* **1977**, *99*, 4256–4259. (c) Dodziuk, H.; Voithenberg, H. v.; Allinger, N. L. *Tetrahedron* **1982**, *38*, 2811–2819. (d) Dodziuk, H. *J. Mol. Struct.* **1974**, *20*, 317–319.

(8) (a) Wells, G. B.; Makinen, M. W. *J. Am. Chem. Soc.* **1988**, *110*, 6343–6352. (b) Mustafi, D.; Sachleben, J. R.; Wells, G. B.; Makinen, M. W. *J. Am. Chem. Soc.* **1990**, *112*, 2558–2566. (c) Wells, G. B.; Mustafi, D.; Makinen, M. W. *J. Am. Chem. Soc.* **1990**, *112*, 2566–2574. (d) Joela, H.; Mustafi, D.; Fair, C. C.; Makinen, M. W. *J. Phys. Chem.* **1991**, *95*, 9135–9144.

(9) (a) Davis, T. D.; Christoffersen, R. E.; Maggiora, G. M. *J. Am. Chem. Soc.* **1975**, *97*, 1347–1354. (b) Hayat, H.; Silver, B. L. *J. Phys. Chem.* **1973**, *77*, 72–78.

- I. SL - C<sup>α</sup>H O  
 Ia. SL - C<sup>α</sup>D O  
 II. SL - C<sup>α</sup>H = C<sup>β</sup>H - C<sup>γ</sup>O O C<sub>2</sub>H<sub>5</sub>  
 IIa. SL - C<sup>α</sup>D = C<sup>β</sup>H - C<sup>γ</sup>O O C<sub>2</sub>H<sub>5</sub>  
 III. SL - C<sup>α</sup>H = C<sup>β</sup>H - C<sup>γ</sup>O O H  
 IIIa. SL - C<sup>α</sup>D = C<sup>β</sup>H - C<sup>γ</sup>O O H  
 IV. SL - C<sup>α</sup>H = C<sup>β</sup>H - C<sup>γ</sup>H O  
 IVa. SL - C<sup>α</sup>D = C<sup>β</sup>H - C<sup>γ</sup>H O  
 IVb. SL - C<sup>α</sup>H = C<sup>β</sup>H - C<sup>γ</sup>D O  
 V. SL - C<sup>α</sup>H = C<sup>β</sup>H - C<sup>γ</sup>H = C<sup>δ</sup>H - C<sup>ε</sup>O O C<sub>2</sub>H<sub>5</sub>  
 Va. SL - C<sup>α</sup>H = C<sup>β</sup>H - C<sup>γ</sup>D = C<sup>δ</sup>H - C<sup>ε</sup>O O C<sub>2</sub>H<sub>5</sub>  
 VI. SL - C<sup>α</sup>H = C<sup>β</sup>H - C<sup>γ</sup>H = C<sup>δ</sup>H - C<sup>ε</sup>O O H  
 VIa. SL - C<sup>α</sup>H = C<sup>β</sup>H - C<sup>γ</sup>D = C<sup>δ</sup>H - C<sup>ε</sup>O O H  
 VII. SL - C<sup>α</sup>H = C<sup>β</sup>H - C<sup>γ</sup>H = C<sup>δ</sup>H - C<sup>ε</sup>H O

**Figure 1.** Spin-label compounds of formyl, propenoyl, and pentadienoyl derivatives and their deuterated analogues (I–VII) that were synthesized for ENDOR studies. SL refers to the 2,2,5,5-tetramethyl-1-oxypyrrolinyl moiety. In the atomic numbering scheme for the side chains, we designate atom positions alphabetically, using Greek letters, starting with the carbon atom attached to the spin-label moiety. The positions of deuterium substitutions are indicated by D rather than <sup>2</sup>H.

with respect to the unpaired electron. The ENDOR-determined electron–proton separations are then used as constraints for torsion angle search calculations to define molecular conformation.

In this study, the ENDOR results show that the polyene side chain has a completely planar *trans* conformation. In the aldehyde derivatives, the carbonyl group assumes an *s-trans* conformation with respect to the alkene chain, while in the acid and ester derivatives the carbonyl group has an *s-cis* conformation. We also provide a detailed description of three-dimensional structures of these molecules. The different orientations of the carbonyl group in these polyene compounds are explained on the basis of steric and dipolar interactions and the  $\pi$ -electron conjugation of the olefinic groups within the side chain with the terminal carbonyl or carboxyl group.

## Experimental Procedures

**General Materials.** The parent spin-label 2,2,5,5-tetramethyl-1-oxypyrrolone-3-carboxylic acid was obtained by hydrolysis of 2,2,5,5-tetramethyl-1-oxypyrrolone-3-carboxamide (Aldrich Chemical Co., Inc., Milwaukee, WI 53233) according to the method of Rozantsev.<sup>11</sup> Sodium ethoxide was prepared immediately before use by dissolving clean sodium metal in absolute ethanol and evaporating the solvent. Deuterated solvents (>99.5% <sup>2</sup>H) were obtained from Cambridge Isotope Laboratories, Inc. (Woburn, MA 01801) or from Aldrich. THF was refluxed and distilled over lithium aluminum hydride and stored over molecular sieves. All reagents were of analytical reagent grade unless otherwise described. Deionized distilled water was used throughout.

**Chromophoric Nitroxyl Spin-Labels.** In Figure 1 are illustrated the structural formulae of the spin-label compounds I–VII synthesized and characterized in this investigation. The syntheses of these compounds are outlined below, and the analytical data defining their composition are summarized in Table I. Compounds I–IV have been synthesized earlier

by others.<sup>12,13</sup> We prefer the procedures presented in this article for the coordinated syntheses of I–VII since they provide a convenient means to introduce selective deuterium substitutions. Mass spectrometric analyses were carried out by Dr. Steven Mullen in the School of Chemical Sciences at the University of Illinois at Urbana-Champaign. Elemental composition was determined by Midwest Microlab, Indianapolis, IN 46250.

**3-Formyl-2,2,5,5-tetramethyl-1-oxypyrrolone (I).** To a suspension of 2,2,5,5-tetramethyl-1-oxypyrrolone-3-carboxylic acid (9.9 g, 54 mmol) in 100 mL of dry benzene maintained at 6–8 °C was added 20 mL of benzene containing 4.4 mL (61 mmol) of multiply distilled, colorless SOCl<sub>2</sub>. To this mixture was added dropwise 25 mL of benzene containing 4.4 mL (54 mmol) dry pyridine over a period of 5 min with vigorous stirring. The mixture was then brought to room temperature, and the pyridinium chloride precipitate was removed by suction filtration. Benzene (250 mL) was added to the orange filtrate, and the solution was reduced to ~20 mL in volume by evaporation *in vacuo*. Residual pyridinium chloride precipitate was removed by gravity filtration.

The filtrate was evaporated *in vacuo* to obtain a dark red oil, 100 mL of THF was added, and the mixture was cooled to –70 °C under a nitrogen atmosphere. A mixture of lithium tris(*tert*-butoxy)aluminum hydride (LiAlH(O-*t*Bu)<sub>3</sub>, 13.8 g, 54 mmol, Aldrich) in 100 mL of THF was added dropwise over 1 h with stirring. The mixture was stirred for one more hour at –70 °C, brought to room temperature over a period of 1 h, and poured over ~800 mL of crushed ice. The precipitate was separated by suction filtration and washed thoroughly with ethyl acetate. The filtrate was extracted with ethyl acetate until the extracts were colorless, and the combined organic extracts and washings were dried over MgSO<sub>4</sub>. After evaporation *in vacuo* to a yellow oil, ~100 mL of pentane was added. Evaporation *in vacuo* of the pentane produced yellow crystals.

The crude aldehyde was dissolved in 2 L of boiling water, and the solution was quickly cooled to room temperature in an ice bath. Insoluble material was removed by suction filtration. The aqueous filtrate was extracted with ethyl acetate until the extracts were colorless, and the combined extracts evaporated to dryness, whereupon 10 mL of water was added to the solid residue. The mixture was shaken with successive 200-mL portions of pentane, and the pentane layers were decanted off. This procedure was repeated until the pentane layer was colorless. The combined pentane extracts, totaling approximately 1500 mL, were washed with 5 mL of 5% NaHCO<sub>3</sub> and 5 mL of water, dried over MgSO<sub>4</sub>, and evaporated to dryness. Recrystallization from pentane at –20 °C gave 4.52 g (50% yield) of the purified aldehyde I. The mass spectrum of I showed an *m/e* ratio of 168 with the characteristic breakdown pattern of an oxypyrrolinyl spin-label species.<sup>14</sup>

3-[(<sup>2</sup>H)formyl]-2,2,5,5-tetramethyl-1-oxypyrrolone (Ia) was synthesized according to the method for I, except that the acid chloride obtained from 5.2 g (28 mmol) of the starting spin-label acid was reduced with lithium tris(*tert*-butoxy)aluminum deuteride (Morton Thiokol, Inc., Danvers, MA 01923). No evidence of the presence of I was detected by mass spectrometric analysis of Ia.

**Ethyl 3-(2,2,5,5-Tetramethyl-1-oxypyrrolinyl)-2-propenoate (II).** A solution of 4.3 g (26 mmol) of I in 25 mL of ethyl acetate was added dropwise with vigorous stirring over a period of 2 h to a suspension of 20.7 g (304 mmol) of freshly prepared sodium ethoxide in 50 mL of ethyl acetate maintained at 0 °C. The mixture was stirred two more hours at 0 °C and brought to room temperature over 60 min. Glacial acetic acid was added dropwise with stirring until neutralization of the reaction mixture was confirmed by testing with moistened pH paper. Approximately 250 mL of water was added, the ethyl acetate layer was separated, and the aqueous layer was extracted with ethyl acetate until the extracts were colorless. The combined organic extracts were washed twice with one-half volume of HCl, once with one-half volume of water, twice with one-half volume of 5% w/v NaHCO<sub>3</sub>, and once more with one-half volume of water, dried over MgSO<sub>4</sub>, and evaporated to dryness. Recrystallization from ether at –20 °C gave 5.1 g (83% yield) of pure product.

For ethyl 3-(2,2,5,5-tetramethyl-1-oxypyrrolinyl)-2-(3-<sup>2</sup>H)propenoate (IIa), the procedure was exactly the same as for compound II, with the use of 1.9 g (11.2 mmol) of Ia as the starting material, giving 2.1 g (79% yield) of the deuterated ester. No evidence of the presence of II was detected by mass spectrometric analysis.

(12) Koch, T. R.; Kuo, L. C.; Douglas, E. G.; Jaffer, S.; Makinen, M. W. *J. Biol. Chem.* **1979**, *254*, 12310–12313.

(13) (a) Hideg, K.; Hankovszky, H. O.; Lex, L.; Kulcsár, Gy. *Synthesis* **1980**, 911–914. (b) Hideg, K.; Hankovszky, H. O. In *Spin-Labeling: Theory and Applications*; Berliner, L. J., Reuben, J., Eds.; *Biol. Magn. Reson.* **1989**, *8*, 427–488.

(14) Andersson, B. A.; Fölsch, G. *Chem. Scr.* **1972**, *2*, 21–24.

(10) Mustafi, D.; Joela, H.; Makinen, M. W. *J. Magn. Reson.* **1991**, *91*, 497–504.

(11) Rozantsev, E. G. *Free Nitroxyl Radicals*; Plenum Press: New York, 1970; Chapter 9, pp 203–246.

Table I. Summary of Analytical Results Characterizing Purified, Crystalline, Synthetic Spin-Label Products

compd	chemical formula	melting point (°C)	elemental analysis calcd (found)			<i>m/e</i>	$\lambda_{\max}$ (nm)	$\epsilon$ (M <sup>-1</sup> cm <sup>-1</sup> )
			C	H	N			
I	C <sub>9</sub> H <sub>14</sub> NO <sub>2</sub>	79.5–80.5	64.26 (63.23)	8.39 (8.31)	8.33 (8.08)	168	227	17 829
Ia	C <sub>9</sub> H <sub>13</sub> DNO <sub>2</sub>	79.5–80.5	63.88 (63.79)	8.93 (8.70)	8.28 (8.28)	169		
II	C <sub>13</sub> H <sub>20</sub> NO <sub>3</sub>	82.5–83.5	65.55 (65.79)	8.40 (8.30)	5.88 (5.80)	238	261	23 678
IIa	C <sub>13</sub> H <sub>19</sub> DNO <sub>3</sub>	82.5–83.5	65.25 (65.23)	8.84 (8.61)	5.86 (6.03)	239		
III	C <sub>11</sub> H <sub>16</sub> NO <sub>3</sub>	194.5 dec	62.84 (62.86)	7.67 (7.63)	6.66 (6.72)	210	253	19 712
IIIa	C <sub>11</sub> H <sub>15</sub> DNO <sub>3</sub>	194.5 dec	62.54 (62.76)	8.11 (7.96)	6.63 (6.65)	211		
IV	C <sub>11</sub> H <sub>16</sub> NO <sub>2</sub>	98.0–98.6	68.01 (68.10)	8.30 (8.31)	7.21 (7.15)	194	274	27 141
IVa	C <sub>11</sub> H <sub>15</sub> DNO <sub>2</sub>	97.5–98.5	67.67 (67.78)	8.77 (8.50)	7.17 (7.23)	195		
IVb	C <sub>11</sub> H <sub>15</sub> DNO <sub>2</sub>	98.0–98.6						
V	C <sub>15</sub> H <sub>22</sub> NO <sub>3</sub>	92.0–92.6	68.16 (68.09)	8.39 (8.50)	5.30 (5.26)	264	300	41 422
Va	C <sub>15</sub> H <sub>21</sub> DNO <sub>3</sub>	91.0–92.0						
VI	C <sub>13</sub> H <sub>18</sub> NO <sub>3</sub>	179.5–180.0	66.08 (66.07)	7.68 (7.75)	5.93 (5.81)	236	296	39 301
VIa	C <sub>13</sub> H <sub>17</sub> DNO <sub>3</sub>	178.0–179.0						
VII	C <sub>13</sub> H <sub>18</sub> NO <sub>2</sub>	112.5–113.0	70.88 (70.78)	8.24 (8.33)	6.36 (6.29)	220	316	41 685

**3-(2,2,5,5-Tetramethyl-1-oxypyrrolinyl)-2-propenoic Acid (III).** In 400 mL of 0.1 N NaOH was suspended 5.0 g (21 mmol) of II. The mixture was stirred overnight at room temperature and extracted once with ethyl acetate. The aqueous mixture was then acidified to pH 2 by dropwise addition of 2 N HCl. The yellow precipitate was collected by suction filtration, and the filtrate was extracted with ethyl acetate until the extracts were colorless. The precipitate was dissolved in the pooled ethyl acetate extracts, and the mixture was washed with one-half volume of water, dried over MgSO<sub>4</sub>, and evaporated to dryness. Recrystallization from THF/pentane at –20 °C gave 3.9 g (88% yield) of the pure acid.

For 3-(2,2,5,5-tetramethyl-1-oxypyrrolinyl)-2-(3-<sup>2</sup>H)propenoic acid (IIIa), the same synthesis procedure was used as for III, with 2.0 g (8.4 mmol) of IIa as the starting material. This gave 1.5 g (84% yield) of the pure acid. Mass spectrometric analysis indicated no evidence of III.

**3-(2,2,5,5-Tetramethyl-1-oxypyrrolinyl)-2-propenal (IV).** This aldehyde was synthesized from III (2.0 g, 8.4 mmol) according to the procedure described for the synthesis of I. Recrystallization from ether at –20 °C gave 0.49 g (27% yield) of the pure product.

**3-(2,2,5,5-Tetramethyl-1-oxypyrrolinyl)-2-(3-<sup>2</sup>H)propenal (IVa)** was synthesized in the same way as IV, using 1.0 g (4.7 mmol) of IIIa as the starting material to give 0.34 g (37% yield) of the pure aldehyde.

**3-(2,2,5,5-Tetramethyl-1-oxypyrrolinyl)-(1-<sup>2</sup>H)propenal (IVb)** was synthesized from 1.0 g (4.8 mmol) of III according to the procedure for the synthesis of IV, except that LiAl<sup>2</sup>H(O-*t*Bu)<sub>3</sub> was used as the reducing agent. The yield was 0.13 g (14% yield).

**Ethyl 5-(2,2,5,5-Tetramethyl-1-oxypyrrolinyl)-2,4-pentadienoate (V).** This compound was synthesized from IV according to the procedure described for the synthesis of II from I, and an 82% yield was obtained.

**Ethyl 5-(2,2,5,5-tetramethyl-1-oxypyrrolinyl)-2,4-(3-<sup>2</sup>H)pentadienoate (Va)** was synthesized from 0.12 g (0.6 mmol) of IVb using the same procedure as for the synthesis of V to yield 0.1 g (61% yield) of the pure product.

**5-(2,2,5,5-Tetramethyl-1-oxypyrrolinyl)-2,4-pentadienoic Acid (VI).** In 300 mL of 0.1 N NaOH was suspended 3.7 g (14 mmol) of V. The solution was stirred at room temperature for 3 days, after which almost all solid material had dissolved. VI was then isolated and purified according to the procedure described above for the synthesis of III. Recrystallization from ether at –20 °C gave 2.2 g (67% yield) of the pure acid.

**5-(2,2,5,5-Tetramethyl-1-oxypyrrolinyl)-2,4-(3-<sup>2</sup>H)pentadienoic acid (VIa)** was synthesized from 0.09 g (0.3 mmol) of Va using the procedure for the synthesis of VI, yielding 0.02 g (25% yield) of the product.

**5-(2,2,5,5-Tetramethyl-1-oxypyrrolinyl)-2,4-pentadienal (VII).** The acid chloride of VI (0.33 g) was prepared and reduced to the aldehyde according to the procedure described for the synthesis of I. The crude aldehyde was dissolved in 1 L of boiling water, and the mixture was then cooled to room temperature in an ice bath. Insoluble impurities were removed by suction filtration. The aldehyde was further purified by the procedures used in the synthesis of I. Recrystallization from ether at –20 °C gave 0.12 g (39% yield) of the pure aldehyde.

**EPR and ENDOR.** ENDOR spectra were recorded with the sample at 20 K with an X-band Bruker ER200D spectrometer equipped with an Oxford Instruments ESR10 liquid helium cryostat and a Bruker digital ENDOR accessory, as previously described.<sup>15</sup> ENDOR spectra were

(15) (a) Mustafi, D.; Makinen, M. W. *Inorg. Chem.* **1988**, *27*, 3360–3368. (b) Yim, M. B.; Makinen, M. W. *J. Magn. Reson.* **1986**, *70*, 89–105.

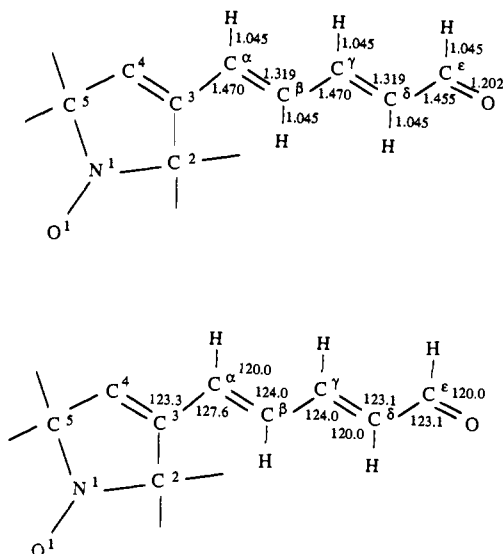


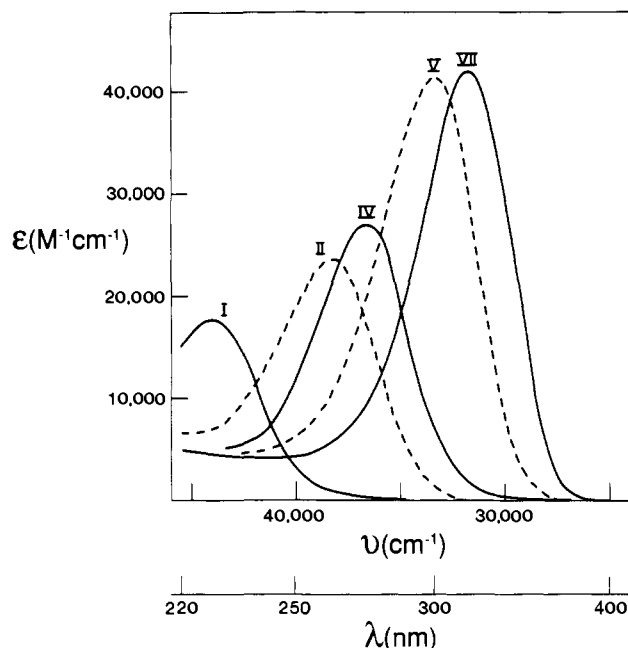
Figure 2. Illustration of the chemical bonding structure and the atomic numbering scheme of 5-(2,2,5,5-tetramethyl-1-oxypyrrolinyl)-2,4-pentadienal (VII). Bond lengths (top) and bond angles (bottom) in the side chain are illustrated in angstroms and degrees, respectively.

recorded in the first-derivative absorption mode with 1.28-mW incident microwave power, 12.5-kHz frequency modulation of the radio frequency (rf) field, and 50-W rf power with ~8-kHz modulation depth of the rf field. The static laboratory magnetic field was not modulated for ENDOR. Spin-labeled derivatives were dissolved for EPR and ENDOR spectroscopy to a concentration of  $5 \times 10^{-3}$  M in (<sup>2</sup>H<sub>5</sub>)methanol or in (<sup>2</sup>H)chloroform: (<sup>2</sup>H<sub>8</sub>)toluene:(<sup>2</sup>H<sub>6</sub>)dimethyl sulfoxide (25:25:50).

**Molecular Modeling.** Atomic coordinates of compounds I–VII were derived on the basis of X-ray-defined molecular fragments. Coordinates of 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-oxypyrrolin-3-carboxamide.<sup>16</sup> The side chains in I–VII were constructed on the basis of bond lengths and bond angles of non-hydrogen atoms of compounds containing similar conjugated side chains, as determined by X-ray diffraction studies.<sup>17</sup> Bond lengths and bond angles, as obtained by this method, for instance, are illustrated for VII in Figure 2. Positions of hydrogen atoms were calculated for idealized geometries with C–H bond lengths of 1.08 and 1.045 Å for *sp*<sup>3</sup> and *sp*<sup>2</sup> hybridized carbons, respectively, as previously described.<sup>8,18</sup> In the side chain the

(16) Turley, J. W.; Boer, F. P. *Acta Crystallogr., Sect. B* **1972**, *28*, 1641–1644.

(17) (a) Nakanishi, H.; Ueno, K.; Sasada, Y. *Acta Crystallogr., Sect. B* **1978**, *34*, 2209–2214. (b) Becher, J.; Svendsen, E. N.; Simonsen, O. *Tetrahedron* **1977**, *33*, 1481–1482. (c) Leser, J.; Rabinovich, D. *Acta Crystallogr., Sect. B* **1978**, *34*, 2257–2259. (d) Ueno, K.; Nakanishi, H.; Hasegawa, M.; Sasada, Y. *Acta Crystallogr., Sect. B* **1978**, *34*, 2034–2035. (e) Hamanaka, T.; Mitsui, T.; Ashida, T.; Kakudo, M. *Acta Crystallogr., Sect. B* **1972**, *28*, 214–222. (f) Kashino, S.; Haisa, M. *Acta Crystallogr., Sect. B* **1980**, *36*, 346–353.



**Figure 3.** Ultraviolet absorption spectra of I, II, IV, V, and VII. The spectra of the three aldehydes are indicated by solid lines, and those of the two esters are indicated by dashed lines. For each spectrum a 0.020-mL aliquot of a stock methanolic solution of the spin-label was diluted in 0.1 M NaCl buffered to pH 7.0 with 0.01 M sodium cacodylate. The absorption spectra were collected with a Cary 15 recording spectrophotometer modified by On-Line Instrument Systems, Inc. (Jefferson, GA 30549) for microprocessor-controlled data acquisition.

mean values of the C–C–H bond angles opposite the single and double bonds are 120° and 116°, respectively.

In Figure 2, the atomic numbering scheme is also shown for purposes of discussing molecular modeling results. To avoid ambiguity in discussing both ENDOR and molecular modeling results, we designate atom positions as  $\alpha$ ,  $\beta$ , ..., starting from the first carbon atom of the side chain substituent bonded to the C(3) position of the spin-label oxypyrrolinyl ring.

Molecular modeling was carried out with the programs FRODO<sup>19</sup> and SYBYL,<sup>20</sup> as previously described.<sup>8</sup> The basic elements and philosophy underlying the use of the program package SYBYL have been described by Naruto et al.,<sup>20a</sup> and parameters for van der Waals contact radii were those of Iijima et al.<sup>20b</sup> With this program package, a systematic conformational analysis was carried out with SEARCH, which checks for van der Waals contacts among nonbonded atoms by scanning all possible torsion angles around rotatable bonds and identifies within the van der Waals allowed conformational space those conformations that are compatible with ENDOR-determined electron–nucleus distances together with their respective uncertainties as added constraints. All double bonds in the alkene side chains were treated as freely rotating single bonds to ensure that “frozen” parts of the molecule did not sterically constrain the allowed range of conformations. Calculations were performed in 2° increments of torsion angle rotation. This increment was found as a reasonable compromise between the accuracy of assigning ENDOR-compatible conformations and the central processor time needed for calculations. The effective position of the unpaired spin density of the nitroxyl group as a point dipole has been assigned earlier.<sup>10</sup>

## Results and Discussion

**A. Spectrophotometric Study of Spin-Labeled Compounds.** We have illustrated in Figure 3 the ultraviolet absorption spectra of

(18) (a) Mustafi, D.; Boisvert, W. E.; Makinen, M. W. *Biopolymers* **1990**, *29*, 45–55. (b) Mustafi, D.; Wells, G. B.; Joela, H.; Makinen, M. W. *Free Radical Res. Commun.* **1990**, *10*, 95–101. (c) Mustafi, D.; Makinen, M. W. *Appl. Magn. Reson.* **1992**, *3*, 321–331.

(19) (a) Jones, T. A. In *Computational Crystallography*; Sayre, D., Ed.; Clarendon Press: Oxford, 1982; pp 303–317. (b) Jones, T. A. *Methods Enzymol.* **1985**, *115*, 157–171.

(20) (a) Naruto, S.; Motoc, J.; Marshall, G. R.; Daniels, S. B.; Sofia, M. J.; Katzenellenbogen, J. A. *J. Am. Chem. Soc.* **1985**, *107*, 5262–5270. (b) Iijima, H.; Dunbar, J. B., Jr.; Marshall, G. R. *Proteins: Struct., Funct., Genet.* **1987**, *2*, 330–339.

I, II, IV, V, and VII, and the electronic absorption properties of I–VII are summarized in Table I. For each compound the broad absorption band can be directly attributed to  $\pi$ ,  $\pi^*$  transitions. The maximum absorption of I at 227 nm is shifted approximately 45 nm to longer wavelength from the position of peak absorption of a saturated aldehyde. It is of interest to note that comparison of the peak absorptions of I, IV, and VII indicates that addition of each olefinic group shifts  $\lambda_{\text{max}}$  to longer wavelength by about 45 nm.

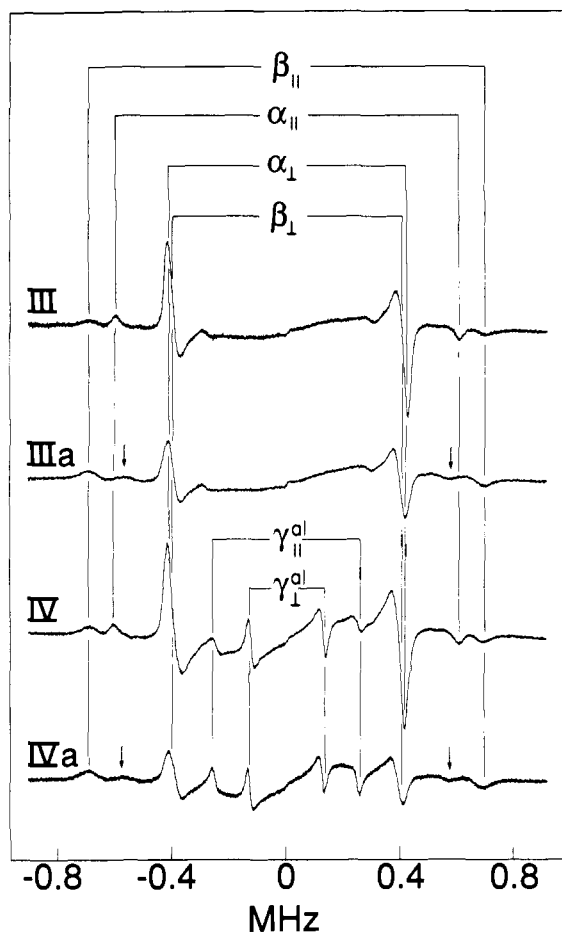
Both the positions of peak absorption and the values of the maximum extinction coefficients of the three spin-label aldehydes I, IV, and VII are in excellent agreement with those of corresponding polyene aldehydes, e.g.,  $\text{CH}_3-(\text{CH}=\text{CH})_n-\text{CHO}$ , which are of a planar *trans* conformation.<sup>21</sup> This observation suggests that the spin-label derivatives probably also exhibit a *trans* structure. Structural isomers having a *cis* conformation would be expected to exhibit maximum extinction at shorter wavelengths and with lower intensity. We have also collected the absorption spectra of these derivatives in a first- and a second-derivative mode<sup>22</sup> and have found that the broad spectral bands of all compounds I–VII were comprised of a single component. Through the analysis of ENDOR results given below, we shall demonstrate that all of the compounds listed in Table I exhibit only planar *trans* conformations.

**B. Assignment of ENDOR Resonances and Estimation of Electron–Proton Distances. 1. ENDOR of Spin-Labeled Propenoic Acid and Propenal.** We have previously outlined the stratagem of employing the magnetic field dependence of the EPR absorption of nitroxyl spin-labels for selection of molecular orientation in ENDOR studies.<sup>8,10,18</sup> The EPR spectrum of nitroxyl spin-labels is dominated by anisotropic hf interactions of the <sup>14</sup>N nucleus of the nitroxyl group, and the spectrum in frozen solutions is a composite of three sets of spectral components that correspond to the  $m_I = +1, 0,$  and  $-1$  states of the <sup>14</sup>N nucleus. The intense central feature of the EPR spectrum arises predominantly from molecules with  $m_I = 0$ , while the low- and high-field features due to the  $A_z$  hf component of  $m_I = +1$  and  $-1$ , respectively, are well separated. For this reason, microwave power saturation of the central feature of the EPR spectrum of a spin-label in frozen solutions selects for ENDOR essentially all orientations of the spin-label with respect to the laboratory magnetic field, while saturation of the low- or high-field region selects molecules for which the five-membered oxypyrrolinyl ring is perpendicular to the applied field. When  $H_0$  is set to the low-field turning point of the EPR spectrum (termed setting A), only the perpendicular principal hfc component  $A_{\perp}$  is observed. When  $H_0$  is set to the central region of the EPR spectrum (termed setting B), both the parallel and the perpendicular principal hfc components ( $A_{\parallel}$  and  $A_{\perp}$ ) are observed. For purposes of brevity, we present ENDOR spectra collected under setting B of the static laboratory magnetic field since the perpendicular hfc components identified under setting A also appear in the B setting spectra. However, assignments of all ENDOR absorptions presented in this study were made on the basis of analyses of spectra collected for both A and B settings.

Figure 4 illustrates proton ENDOR spectra of III–IVa. The hfc components are assigned as described above according to the stratagem described for selection of molecular orientation. Since the carboxylic proton exchanges rapidly with solvent deuterons, the ENDOR resonance features of this proton are absent. The individual resonance features of the  $\alpha$  and  $\beta$  protons in the spectra of III and IV can be identified by selective deuteration, as seen in Figure 4. Figure 5 illustrates proton ENDOR spectra of III

(21) (a) Dyer, J. R. *Applications of Absorption Spectroscopy of Organic Compounds*; Prentice-Hall, Inc.: New Jersey, 1965; Chapter 2, pp 4–21. (b) Pippen, E. L.; Nonaka, M. *J. Org. Chem.* **1958**, *23*, 1580–1582.

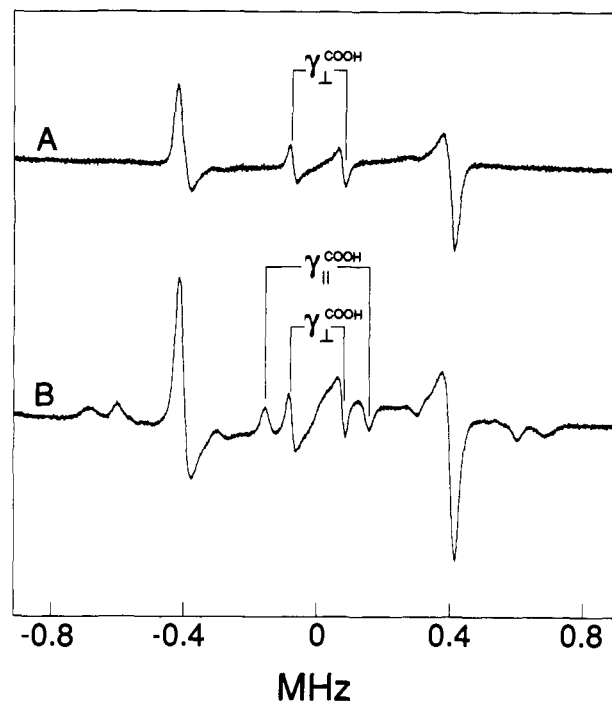
(22) (a) Stauffer, F. R.; Sakai, H. *Appl. Opt.* **1968**, *7*, 61–65. (b) Talsky, G.; Mayring, L.; Kreuzer, H. *Angew. Chem., Int. Ed. Engl.* **1978**, *17*, 532–533. (c) O'Haver, T. C.; Begley, T. *Anal. Chem.* **1981**, *53*, 1876–1878.



**Figure 4.** Proton ENDOR spectra of III–IVa in ( $^2\text{H}_4$ )methanol with  $\text{H}_0$  at setting B. For each proton two pairs of resonance features are seen and are assigned to parallel and perpendicular hfc components. The weak features indicated by arrows are assigned to the parallel hfc component of the vinylic proton of the oxypyrrolinyl ring (see ref 18b). The line pairs are labeled according to the atomic numbering scheme (see Figure 1). An additional pair of resonance features is observed near those assigned to the perpendicular hfc components of  $\text{H}^\alpha$  and  $\text{H}^\beta$ . The relative intensity of these additional features was observed to vary for different synthesis lots. Since the ENDOR features of  $\text{H}^\alpha$  and  $\text{H}^\beta$  are identified on the basis of selective deuteration, we ascribe, therefore, these additional features to impurities.

with  $\text{H}_0$  at settings A and B, in the perdeuterated dimethyl sulfoxide:chloroform:toluene solvent mixture. The ENDOR features for  $\text{H}^\alpha$  and  $\text{H}^\beta$  and their line splittings in this aprotic ternary solvent system are identical to those observed in the polar solvent methanol and, therefore, are not labeled. However, in this case, use of an aprotic solvent system provides an opportunity to detect the resonance features of the  $\text{H}^{\text{COOH}}$  that normally is rapidly exchanged with deuterons in ( $^2\text{H}_4$ )methanol. In Figure 5, two well-resolved pairs of features near the free proton frequency are assigned to the parallel and perpendicular hfc components of the carboxylic acid proton.

In Table II we have summarized the observed values of the parallel and perpendicular hfc components of protons at the  $\alpha$ ,  $\beta$ , and  $\gamma$  positions in I, III, and IV. The detection of only two pairs of resonance features for each proton requires that the observed ENDOR splittings correspond to axially symmetric principal hfc components. Under the conditions that  $|A_{\text{iso}}| \ll |A_{\parallel}|$ ,  $|A_{\perp}|$  and  $A_{\parallel}^{\text{D}} > 0 > A_{\perp}^{\text{D}}$ , the dipolar hfc components  $A_{\parallel}^{\text{D}}$  and  $A_{\perp}^{\text{D}}$  have been calculated under the constraint  $(A_{\parallel} + 2A_{\perp}) = 3A_{\text{iso}}$ .<sup>8</sup> We have previously shown that the value and relative sign of  $A_{\text{iso}}$  for nitroxides in frozen solution obtained by this method correspond exactly to those determined directly by ENDOR and TRIPLE resonance in solution.<sup>10</sup>



**Figure 5.** Proton ENDOR spectra of compound III in ( $^2\text{H}_6$ )DMSO: ( $^2\text{H}$ )chloroform:( $^2\text{H}_8$ )toluene with  $\text{H}_0$  at settings A (upper spectrum) and B (lower spectrum). The ENDOR line splittings for the  $\gamma^{\text{COOH}}$  proton are identified in the stick diagram as  $\gamma_{\parallel}^{\text{COOH}}$  and  $\gamma_{\perp}^{\text{COOH}}$ . The ENDOR features for the  $\alpha$  and  $\beta$  protons are not labeled (see Figure 4).

In Table II we have also listed the values of isotropic and dipolar hfc components for each proton together with corresponding electron–proton separations  $r$  calculated on the basis of the dipolar equation.

## 2. ENDOR of Spin-Labeled Pentadienoic Acid and Pentadienal.

In Figure 6 the proton ENDOR spectra of VI–VII in ( $^2\text{H}_4$ )methanol are illustrated. The identification of separate resonance features for  $\text{H}^\alpha$  and  $\text{H}^\beta$  was made on the basis of selective deuteration, as described for spin-labeled propenoyl derivatives in Figure 4. The principal hfc components for the carboxylic acid proton of VI are assigned from ENDOR spectra of VI in the perdeuterated ternary solvent system (data not shown). Since the carboxylic acid proton exchanges with solvent deuterons in ( $^2\text{H}_4$ )methanol, four line pairs for  $\text{H}^\gamma$  and  $\text{H}^\delta$  should be observed in the ENDOR spectrum of VI near the free proton frequency. In the spectrum of VI, we have identified both parallel hfc components for  $\text{H}^\gamma$  and  $\text{H}^\delta$ , but their perpendicular features overlap. However, both sets of hfc components for  $\text{H}^\gamma$  and  $\text{H}^\delta$  were assigned by comparing the ENDOR spectra of VI and VIa. ENDOR absorption features of compounds VII and VI are identical except that in the spectrum of VII two additional sets of resonance features are observed near the free proton frequency. These features are assigned to the aldehydic proton and are labeled as  $\epsilon_{\parallel}^{\text{al}}$  and  $\epsilon_{\perp}^{\text{al}}$ .

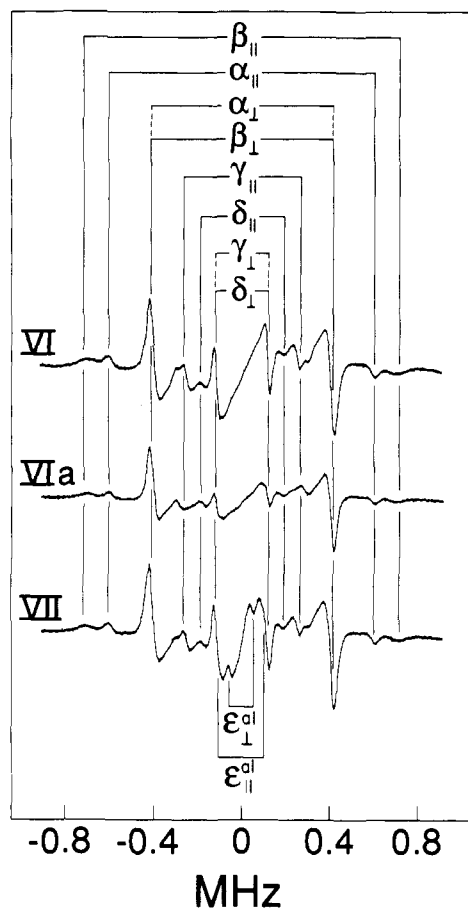
We have summarized in Table III the values of the principal hfc components and electron–proton separations for each type of proton in compounds VI and VII.

**C. Conformations of Spin-Labeled Olefinic Carbonyl Compounds.** To determine the conformations of I–VII, we have carried out a computational analysis based on torsion angle search calculations. The general methodology of application of torsion angle search calculations to define molecular conformation constrained by ENDOR-determined electron–proton distances has been described in earlier publications.<sup>8,18</sup> The molecular models for these calculations were constructed as described in the Experimental Procedures. The torsion angle SEARCH calculations were carried out within van der Waals hard-sphere

**Table II.** Summary of hfc Components and Estimated Electron-Proton Distances in 3-Formyl-2,2,5,5-tetramethyl-1-oxypyrrolone (I), 3-(2,2,5,5-Tetramethyl-1-oxypyrrolinyl)-2-propenoic Acid (III), and 3-(2,2,5,5-Tetramethyl-1-oxypyrrolinyl)-2-propenal (IV)

proton <sup>a</sup>	compd	hfc component (MHz)					<i>r</i> <sup>b</sup> (Å)
		<i>A</i> <sub>  </sub>	<i>A</i> <sub>⊥</sub>	<i>A</i> <sub>iso</sub>	<i>A</i> <sub>  </sub> <sup>D</sup>	<i>A</i> <sub>⊥</sub> <sup>D</sup>	
α <sup>al</sup>	I	1.064	0.958	-0.284	1.348	-0.674	4.91 ± 0.02
α	III, IV	1.214	0.809	-0.135	1.349	-0.674	4.91 ± 0.02
β	III-IVb	1.394	0.794	-0.065	1.459	-0.729	4.76 ± 0.02
γ <sup>COOH</sup>	III	0.313	0.156	0.000	0.313	-0.156	7.98 ± 0.07
γ <sup>al</sup>	IV	0.515	0.262	-0.003	0.518	-0.259	6.75 ± 0.04

<sup>a</sup> ENDOR line pairs for each proton of compounds III and IV are assigned in Figures 4 and 5. <sup>b</sup> Uncertainty in frequency of 0.008–0.012 MHz due to the line width of each absorption is included in the calculation of electron-proton distances.



**Figure 6.** Proton ENDOR spectra of compounds VI–VII in (<sup>2</sup>H<sub>4</sub>)methanol with H<sub>0</sub> at setting B. The ENDOR splittings that correspond to principal hfc components for each type of proton are identified by the stick diagrams. The line pairs are labeled according to the atomic numbering scheme (see Figure 2).

limits<sup>20</sup> constrained by the ENDOR-determined electron-proton separations listed in Tables II and III.

In Table IV we have summarized the values of dihedral angles characterizing compounds I–VII and determined by ENDOR-constrained torsion angle calculations. The distance constraint to the aldehydic proton of I resulted in a value of 180 ± 12° for the [C(4)=C(3)–C<sup>α</sup>=O] dihedral angle. The electron-proton distance of 4.91 ± 0.02 Å in I requires that the C=O group can assume only an *s-trans* orientation with respect to the double bond of the oxypyrrolinyl ring. An uncertainty of 12° in the dihedral angle is estimated on the basis of the uncertainty of ±0.02 Å in *r* (Table II).

Calculations for the spin-labeled propenoyl ester, acid, and aldehyde derivatives (II–IV) with ENDOR-determined distance constraints to the H<sup>α</sup> and H<sup>β</sup> showed that the C<sup>α</sup>=C<sup>β</sup> double bond is essentially coplanar with the pyrrolinyl ring. Moreover, the results showed the olefinic side chain to have a planar *trans* conformation, as measured by the [C(4)=C(3)–C<sup>α</sup>=C<sup>β</sup>] di-

hedral angle listed in Table IV. On the other hand, for the propenoyl acid derivative III, the single distance constraint of 7.98 ± 0.07 Å to the γ<sup>COOH</sup> proton is associated with a large conformational space around the [C<sup>α</sup>=C<sup>β</sup>–C<sup>γ</sup>–O(H)] dihedral angle (180 ± 60°). This circumstance arises because rotation around the C<sup>β</sup>–C<sup>γ</sup> bond keeps the carboxylic acid proton nearly equidistant from the nitroxyl group. A similar observation was reported for the carboxylic proton of oxypyrrolinyl spin-labeled derivative of L-phenylalanine.<sup>8d</sup> Nonetheless, as seen in Table IV, it is clear that the C=O group in the propenoyl acid takes the *s-cis* conformation with respect to the C<sup>α</sup>=C<sup>β</sup> double bond, and the *s-trans* conformation ([C<sup>α</sup>=C<sup>β</sup>–C<sup>γ</sup>–OH] = 0°) is ruled out. Furthermore, the distance constraint to the γ<sup>COOH</sup> proton places the carboxylic acid group into a classical Z conformation ([C<sup>β</sup>–C<sup>γ</sup>–O–H] = 180 ± 20°), and the *E* conformation, requiring a [C<sup>β</sup>–C<sup>γ</sup>–O–H] dihedral angle of ~0°, is ruled out. For esters, a similar conformation, *trans, s-cis* with a classical Z rotamer of the ester group, is also described.<sup>18a</sup>

On the other hand, the ENDOR distance constraint to the γ<sup>al</sup> proton of the aldehyde IV places the C=O group in the *s-trans* conformation ([C<sup>α</sup>=C<sup>β</sup>–C<sup>γ</sup>=O] = 180 ± 15°), and the *s-cis* conformation is not compatible with the ENDOR data. However, the ENDOR-constrained torsion angle calculations yielded a second conformer as compatible with this distance constraint, indicated as conformer 2 in Table IV, for which the dihedral angle [C<sup>α</sup>=C<sup>β</sup>–C<sup>γ</sup>=O] = 60 ± 15°. This conformer would place the carbonyl group just out of plane with the C<sup>α</sup>=C<sup>β</sup> bond. With respect to the *s-trans* conformer 1, conformer 2 would have a high potential energy with consequent low population in solution.<sup>23</sup>

The conformational analyses of the spin-labeled pentadienyl ester, acid, and aldehyde derivatives V–VII are similar to those of the corresponding propenoyl derivatives. As seen in Table IV, torsion angle calculations for the acid and ester derivatives yielded a single conformation that corresponds to an essentially all planar *trans, s-cis* structure with a classical Z rotamer for the carboxylate group. For the aldehyde VII, on the other hand, two distinct conformers are found, listed as conformers 1 and 2 in Table IV. Conformer 1 has an all planar *trans, s-trans* structure. In Figure 7 we compare the ENDOR-constrained conformation (conformer 1) of VII with an ideal, fully planar, all *trans, s-trans* structure. In the ENDOR-constrained structure of conformer 1, the side chain deviates only ~15° from the plane of the oxypyrrolinyl ring. In conformer 2 of VII, both the C<sup>γ</sup>=C<sup>β</sup> and the C=O group would be ~40–60° out of the plane containing the oxypyrrolinyl ring and the C<sup>α</sup>=C<sup>β</sup> olefinic group. This out-of-plane conformation would disrupt π-conjugation and would consequently be less favored energetically. It is, therefore, probable that conformer 2 is not populated to any measurable extent for the molecule in solution and that this conformation merely represents a case in which the electron–H<sup>α</sup> distance is mathematically accommodated by the torsion angle search

(23) Estimates of the barrier to rotation in acrolein show that an *s-cis* or *gauche* conformer is about 2.5–5.0 kcal/mol above that of an *s-trans* conformer.<sup>24</sup>

(24) Wagner, R.; Fine, J.; Simmons, J. W.; Goldstein, J. H. *J. Chem. Phys.* 1957, 26, 634–637.

**Table III.** Summary of hfc Components and Estimated Electron-Proton Distances in 5-(2,2,5,5-Tetramethyl-1-oxypyrrolinyl)-2,4-pentadienoic Acid (VI) and 5-(2,2,5,5-Tetramethyl-1-oxypyrrolinyl)-2,4-pentadienal (VII)

proton <sup>a</sup>	compd	hfc component (MHz)					$r^b$ (Å)
		$A_{\parallel}$	$A_{\perp}$	$A_{iso}$	$A_{\parallel}^D$	$A_{\perp}^D$	
$\alpha$	VI-VII	1.208	0.811	-0.138	1.346	-0.673	4.91 ± 0.02
$\beta$	VI-VII	1.410	0.821	-0.077	1.487	-0.744	4.73 ± 0.02
$\gamma$	VI-VII	0.519	0.230	0.020	0.499	-0.250	6.83 ± 0.05
$\delta$	VI-VII	0.395	0.238	-0.027	0.422	-0.211	7.20 ± 0.06
$\epsilon^{COOH}$	VI	0.142	0.076	-0.003	0.145	-0.073	10.30 ± 0.16
$\epsilon^{al}$	VII	0.215	0.112	-0.003	0.218	-0.109	8.99 ± 0.08

<sup>a</sup> ENDOR line pairs for each proton are assigned from ENDOR spectra of compounds VI-VII as in Figure 6. <sup>b</sup> Uncertainty in frequency of 0.010-0.016 MHz due to the line width of each absorption is included in the calculation of electron-proton distances.

**Table IV.** Values of Dihedral Angles of ENDOR-Constrained Conformations of Conjugated Polyene Carbonyl Derivatives of Spin-Labels I-VII

compd	dihedral angle <sup>a</sup>	$\tau$ (deg)	
		conformer 1	conformer 2 <sup>b</sup>
I	[C(4)=C(3)-C <sup><math>\alpha</math></sup> =O]	180 ± 12	
II-IV	[C(4)=C(3)-C <sup><math>\alpha</math></sup> =C <sup><math>\beta</math></sup> ]	168 ± 5	
II-IV	[C(3)-C <sup><math>\alpha</math></sup> =C <sup><math>\beta</math></sup> -C <sup><math>\gamma</math></sup> ]	-163 ± 5	
III	[C <sup><math>\alpha</math></sup> =C <sup><math>\beta</math></sup> -C <sup><math>\gamma</math></sup> -OH]	180 ± 60	
III	[C <sup><math>\beta</math></sup> -C <sup><math>\gamma</math></sup> -O-H]	180 ± 20	
IV	[C <sup><math>\alpha</math></sup> =C <sup><math>\beta</math></sup> -C <sup><math>\gamma</math></sup> =O]	180 ± 15	60 ± 15
V-VII	[C(4)=C(3)-C <sup><math>\alpha</math></sup> =C <sup><math>\beta</math></sup> ]	170 ± 5	
V-VII	[C(3)-C <sup><math>\alpha</math></sup> =C <sup><math>\beta</math></sup> -C <sup><math>\gamma</math></sup> ]	-165 ± 5	
V-VII	[C <sup><math>\alpha</math></sup> =C <sup><math>\beta</math></sup> -C <sup><math>\gamma</math></sup> =C <sup><math>\delta</math></sup> ]	170 ± 5	40 ± 10
V-VII	[C <sup><math>\beta</math></sup> -C <sup><math>\gamma</math></sup> =C <sup><math>\delta</math></sup> -C <sup><math>\epsilon</math></sup> ]	-165 ± 5	
V1	[C <sup><math>\gamma</math></sup> =C <sup><math>\delta</math></sup> -C <sup><math>\epsilon</math></sup> -OH]	180 ± 60	
VI	[C <sup><math>\delta</math></sup> -C <sup><math>\epsilon</math></sup> -O-H]	180 ± 22	
VII	[C <sup><math>\gamma</math></sup> =C <sup><math>\delta</math></sup> -C <sup><math>\epsilon</math></sup> =O]	180 ± 22	55 ± 15

<sup>a</sup> Dihedral angles are designated according to the atomic numbering schemes as in Figures 1 and 2. Starting structures were completely planar *trans* conformations. <sup>b</sup> Dihedral angles not listed for conformer 2 are identical to those for conformer 1.

calculations. A similar result was obtained for a nearly eclipsed conformation of spin-labeled methyl L-tryptophanate.<sup>8c</sup>

In the above torsion angle calculations, the double bonded olefinic groups of the side chain were allowed full rotational freedom and were not constrained to remain planar. It is of interest, therefore, to compare the ENDOR-determined distance constraints with predictions of idealized *cis* and *trans* conformations of each olefinic group in the side chain of VI and VII. In Table V we have summarized electron-proton distances  $r$  in the idealized *cis* and *trans* isomers of spin-labeled pentadienoic acid VI and aldehyde VII and have compared these values with those obtained by ENDOR. As seen in Table V, when both the  $\tau_1$  and  $\tau_3$  dihedral angles are *cis* (i.e., two adjacent double bonds are eclipsed with respect to each other), none of the predicted distances are compatible with the corresponding ENDOR-determined values  $r$ . For conformations in which either the  $\tau_1$  or the  $\tau_3$  dihedral angle is 0°, certain values of  $r$  are within the uncertainties of the ENDOR estimates. However, only a completely planar *trans* structure of the side chain in both VI and

**Table V.** Comparison of Electron-Proton Distances<sup>a</sup> in the *cis* and *trans* Isomers of 5-(2,2,5,5-Tetramethyl-1-oxypyrrolinyl)-2,4-pentadienoic Acid (VI) and 5-(2,2,5,5-Tetramethyl-1-oxypyrrolinyl)-2,4-pentadienal (VII)

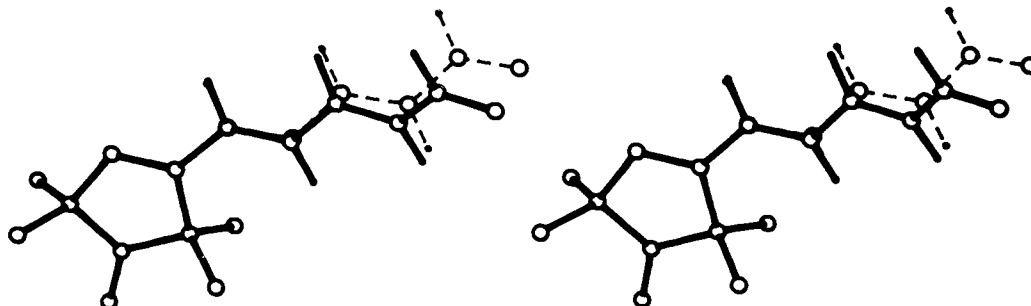
proton <sup>b</sup>	isomer				ENDOR <sup>d</sup>
	A <sup>c</sup>	B <sup>c</sup>	C <sup>c</sup>	D <sup>c</sup>	
$\alpha$	4.54	4.54	4.92	4.92	4.91 ± 0.02
$\beta$	5.44	5.44	4.70	4.70	4.73 ± 0.02
$\gamma$	7.47	6.88	7.00	6.88	6.83 ± 0.05
$\delta$	6.57	7.85	6.87	7.13	7.20 ± 0.06
$\epsilon^{COOH}$	9.96	10.93	10.24	10.42 <sup>e</sup>	10.30 ± 0.16
$\epsilon^{al}$	9.19	9.26	9.06	9.07 <sup>e</sup>	8.99 ± 0.08

<sup>a</sup> Electron-proton distances are given in angstroms. <sup>b</sup> See the atomic numbering scheme in Figure 2 for atomic designations. <sup>c</sup> In isomers A-D,  $\tau_1$ ,  $\tau_2$ ,  $\tau_3$ , and  $\tau_4$  refer to the dihedral angles around the C(3)-C <sup>$\alpha$</sup> , C <sup>$\alpha$</sup> -C <sup>$\beta$</sup> , C <sup>$\beta$</sup> -C <sup>$\gamma$</sup> , and C <sup>$\gamma$</sup> -C <sup>$\delta$</sup>  bonds, respectively. The value of the dihedral angles for a completely planar *trans* conformation of the molecule involving non-hydrogen atoms is 180°. The dihedral angles are indicated in the table only when their values are not 180°. <sup>d</sup> ENDOR-determined  $r$  as listed in Table III. <sup>e</sup> These distances to the acid and the aldehydic protons correspond to conformations where the value of the dihedral angle [C <sup>$\gamma$</sup> =C <sup>$\delta$</sup> -C <sup>$\epsilon$</sup> =O] is 0° (*s-cis*) for VI and 180° (*s-trans*) for VII.

VII (conformer D in Table V) is fully compatible with all of the ENDOR-determined distances.

For the comparison of VI and VII in Table V, it is seen that the acid derivative differs from the aldehyde in the orientation of the C=O group with respect to the alkene side chain. In an *s-trans* conformation of VI, for which the value of the dihedral angle [C <sup>$\gamma$</sup> =C <sup>$\delta$</sup> -C <sup>$\epsilon$</sup> =O] is 180° (or [C <sup>$\gamma$</sup> =C <sup>$\delta$</sup> -C <sup>$\epsilon$</sup> -O(H)] = 0°), the electron-proton distance to H<sup>COOH</sup> would be 10.04 Å. However, in the *s-cis* conformation ([C <sup>$\gamma$</sup> =C <sup>$\delta$</sup> -C <sup>$\epsilon$</sup> =O] = 0°), the distance  $r$  to the H<sup>COOH</sup> is 10.42 Å, in good agreement with the ENDOR-determined value of 10.30 ± 0.16 Å. For VII, on the other hand, the ENDOR-determined distance  $r$  to the aldehydic proton is 8.99 ± 0.08 Å, which is compatible only with an *s-trans* conformation for the C=O group.

**D. Factors Determining the Conformation of the Carbonyl Group in Alkene Aldehydes, Acids, and Esters.** The spin-labeled olefinic carbonyl derivatives described in this investigation have conjugated double bonds. An important consequence of the



**Figure 7.** Stereodiagram comparing the ENDOR-constrained conformation (solid bonds) to the fully planar *trans*, *s-trans* conformation (broken bonds) of spin-labeled pentadienal VII. The ENDOR-constrained structure is drawn according to the mean values of dihedral angles for conformer 1, as listed in Table IV. The ENDOR-significant hydrogen positions for H <sup>$\alpha$</sup> , H <sup>$\beta$</sup> , H <sup>$\gamma$</sup> , H <sup>$\delta$</sup> , and H <sup>$\epsilon$</sup>  are shown in addition to the positions of all non-hydrogen atoms.



**Table VI.** Comparisons of Values of Molar Extinction Coefficients and Positions of Peak Absorptions of Spin-Labeled Olefinic Aldehydes and Corresponding Polyene Aldehydes<sup>a</sup> with Known Planar *trans*, *s-trans* Conformation

spin-labeled compd <sup>b</sup>	$\lambda_{\max}$ (nm)	$\epsilon_{\max}$ (M <sup>-1</sup> cm <sup>-1</sup> )	reference compd	$\lambda_{\max}$ (nm)	$\epsilon_{\max}$ (M <sup>-1</sup> cm <sup>-1</sup> )
SL-CHO	227	17 829	CH <sub>3</sub> -CH=CH-CHO	218	16 500
SL-CH=CH-CHO	274	27 141	CH <sub>3</sub> -(CH=CH) <sub>2</sub> -CHO	270	27 000
SL-(CH=CH) <sub>2</sub> -CHO	316	41 685	CH <sub>3</sub> -(CH=CH) <sub>3</sub> -CHO	312	40 000

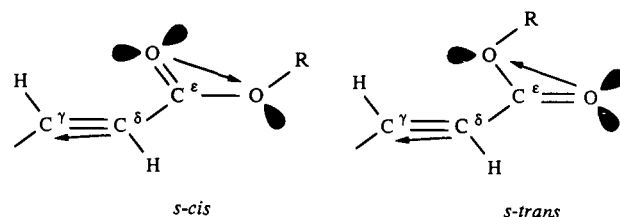
<sup>a</sup> Reference 21. <sup>b</sup> SL refers to the 2,2,5,5-tetramethyl-1-oxypyrrolinyl moiety which contains an olefinic bond that is conjugated to double bonds in the side chain. In this manner the comparison of spin-labeled compounds to each reference compound is made on the basis of identical numbers of olefinic bonds.

delocalization of conjugated  $\pi$ -electrons is a planar conformation of the molecule, which can exist in both *cis* and *trans* diastereomeric forms. The structures of the spin-labeled alkene aldehydes, acids, and esters determined by ENDOR were found to be essentially planar and are almost identical to those of corresponding polyene carbonyl derivatives determined by X-ray diffraction studies.<sup>17</sup> However, it is not immediately apparent why the conformation of the C=O group with respect to the olefinic group is *cis* in the acid and ester derivatives but *trans* in the aldehydes IV and VII.

For the aldehydes, an *s-trans* conformation is favored for three reasons: (1) this conformation has less steric hindrance because the C=O bond is *trans* to the C=C bond; (2) resonance stabilization is much greater for the *trans* form, which can accommodate planarity of the olefinic carbonyl  $\pi$ -electron system; and (3), as we will discuss below, the interaction between the electric dipole of the olefinic group and that of the carbonyl group is more favorable for the *trans* form than for the *cis* form. In Table VI we have compared values of the molar extinction coefficients and positions of peak absorptions of the spin-labeled olefinic aldehydes (I, IV, and VII) and corresponding polyene aldehydes with known planar *trans*, *s-trans* conformations. For compound I, a slightly higher value of both  $\lambda_{\max}$  and  $\epsilon_{\max}$  might be due to the presence of alkyl groups on both vinyl carbons that are adjacent to the C=O group; in the corresponding reference compound only the  $\beta$  position has a methyl group. For the higher homologues IV and VII, for which the alkyl groups are located far from the terminal C=O group, values of  $\lambda_{\max}$  and  $\epsilon_{\max}$  are almost identical to the corresponding values for reference compounds. This comparison indicates that the spin-labeled aldehydes must have a *trans*, *s-trans* structure similar to that of the polyene aldehydes.

The extended  $\pi$ -electron delocalization and conjugation of the carbonyl group into the double bond system in aldehydes are confirmed by measurements of dipole moments and heats of hydrogenation.<sup>3,25</sup> The dipole moments of conjugated olefinic aldehydes are higher than those of corresponding saturated aldehydes,<sup>3,26</sup> indicating a sizable increase in charge separation due to  $\pi$ -electron delocalization. Moreover, the resonance energy of an olefinic aldehyde, as determined for crotonaldehyde (2.9 kcal/mol) from heat of hydrogenation data,<sup>25</sup> suggests that the large increase in stability associated with conjugated alkene aldehydes arises mostly because the conjugated  $\pi$ -electron system is extended from the polyene chain to the carbonyl oxygen.

For olefinic acids and esters, on the other hand, both dipole moment measurements and heat of hydrogenation data suggest that the conjugation does not extend from the alkene chain to the carboxylate group. The  $\pi$ -electron systems of the polyene chain and the carboxyl group remain to a large extent isolated from each other. The dipole moment value of 1.9 D for ethyl sorbate is almost identical to the corresponding value of 1.8 D for ethyl acetate,<sup>3b</sup> indicating that the conjugated polyene carboxylic acids and esters have a less polar structure and that there is little



**Figure 8.** Diagram comparing *s-cis* and *s-trans* structures of the terminal carboxyl portion of spin-labeled pentadienoic acid or ester (R = H or C<sub>2</sub>H<sub>5</sub>). The diagram also illustrates the location of the lone-pair orbitals that are in the plane of the heavy atoms, assuming *sp*<sup>2</sup> hybridization for the ether oxygen atom. The electric dipoles for the carboxylate group and the alkene chain are represented by arrows and are drawn from the negative to the positive end (see text).

**Table VII.** Estimated Interaction Energies of the Dipoles of the Alkene Chain<sup>a</sup> and the Carboxyl Group<sup>b</sup> in Spin-Labeled Pentadienoic Ester V or Acid VI and of the Alkene Chain and the Carbonyl Group<sup>c</sup> in Corresponding Aldehyde VII

compd	conformer	angle <sup>d</sup> (deg)	dipolar energy (kcal/mol)
V, VI	all <i>trans</i> , <i>s-cis</i>	155	-0.270
V, VI	all <i>trans</i> , <i>s-trans</i>	38	0.523
VII	all <i>trans</i> , <i>s-cis</i>	114	-3.427
VII	all <i>trans</i> , <i>s-trans</i>	0	-3.862

<sup>a</sup> The dipole moment value of 1.0 D is calculated for the electric dipole of oxypyrrolinyl-2,4-pentadiene, as described for 1-butene and 2,4-pentadiene.<sup>26</sup> <sup>b</sup> The dipole moment value of 1.8 D is calculated for the carboxylate group that lies at an angle of 39.4° from the C=O bond and almost on the line between the carbonyl oxygen and ester oxygen.<sup>3c</sup> <sup>c</sup> The dipole moment value of 2.4 D for the electric dipole of the carbonyl group is taken from the C=O bond moment.<sup>26</sup> <sup>d</sup> Angle between indicated dipoles.

conjugation of the carboxylate group into the alkene chain. Furthermore, the heat of hydrogenation of vinyl acetate is nearly identical to that of propylene,<sup>25</sup> indicating that the presence of the carboxylate group adjacent to the olefinic group does not contribute to the resonance stabilization. This is supported by the fact that values of  $\lambda_{\max}$  and  $\epsilon_{\max}$  for spin-labeled alkene acids and esters are lower than the corresponding values for aldehydes, as listed in Table I, suggesting almost no or very little  $\pi$ -electron delocalization between the two chromophoric groups in acids or esters.

Unlike conjugated alkene aldehydes, the stability of a particular conformation for alkene acids and esters cannot be rationalized in terms of steric interactions or resonance. Figure 8 illustrates the *s-cis* and the *s-trans* structures of the terminal carboxylate portion of spin-labeled pentadienoic acid or ester. The repulsion between the *2p*<sub>z</sub> orbitals of the olefinic carbons (perpendicular to the plane of the paper, not shown in Figure 8) and the lone-pair orbitals of the carbonyl or the ester oxygen does not favor one conformation over the other. Since, as we have seen, there is little conjugation between the olefinic and the carboxyl  $\pi$ -electron systems, resonance stabilization is also not crucial in determining the conformation. We therefore believe that the interaction between the electrostatic dipoles of the alkene group and the carboxylate group must be the decisive factor in the stabilization of the *s-cis* conformation.

In Table VII, we compare the dipolar interaction energies for *s-cis* and *s-trans* conformers of the acid and ester illustrated in

(25) (a) Dolliver, M. A.; Gresham, T. L.; Kistiakowsky, G. B.; Smith, E. A.; Vaughan, W. E. *J. Am. Chem. Soc.* **1938**, *60*, 440-450. (b) Ingold, C. K. *Structure and Mechanism in Organic Chemistry*; 2nd ed.; Cornell University Press: Ithaca, NY, 1969; Chapter 3, pp 139-142.

(26) Smyth, C. P. *Dielectric Behavior and Structure*; McGraw-Hill: New York, 1955; Chapter 9, pp 260-311.



Figure 8. In the *s-cis* conformation the two dipoles are oriented approximately antiparallel, while in the *s-trans* conformation the dipoles are more nearly parallel. On the basis of calculated dipolar interaction energies alone, an *s-cis* conformation for the acid or the ester is stabilized by 0.8 kcal/mol over an *s-trans* structure, and the population of *s-cis* conformation is expected to be at least 80% of the total at room temperature. In Table VII, we also compare dipolar interaction energies for the two conformers of spin-labeled pentadienal VII. In addition to resonance stabilization and release of steric strain in an *s-trans* conformation of the aldehyde, the estimated dipolar interaction energy also favors this conformation over *s-cis* by 0.4 kcal/mol.

**E. Concluding Remarks.** The results presented here demonstrate the usefulness of ENDOR as an accurate, noncrystallographic method for determining the structures of molecules immobilized by freezing of the solution. In this investigation we have provided detailed three-dimensional structures of conjugated polyene carbonyl derivatives of nitroxyl spin-labels. We also rationalized the origin of different orientations of the carbonyl group with respect to the polyene chain in aldehydes, acids, and esters on the basis of resonance and  $\pi$ -electron conjugation and on the basis of steric and dipolar interactions. Because of the extended  $\pi$ -electron delocalization from the oxypyrrrolinyl ring to the terminal carbonyl group, each of the spin-labeled compounds studied here exhibits a single conformation in solution. In Table VIII, we summarize several key factors that favor a particular conformation (*s-trans* or *s-cis*) of the conjugated polyene

**Table VIII.** Factors That Favor a Particular Conformation of the Terminal Functional Group in Polyene Aldehydes, Acids, and Esters

factors	aldehyde	acid/ester <sup>a</sup>
steric hindrance	<i>s-trans</i>	
resonance energy	<i>s-trans</i>	
dipolar interaction	<i>s-trans</i>	<i>s-cis</i>

<sup>a</sup> In acids and esters, the steric hindrance and resonance energy do not favor one conformation over the other.

aldehydes, acids, and esters. On the other hand, in the accompanying article<sup>27</sup> we provide, with use of a spin-labeled propenol derivative, direct experimental evidence of multiple conformations of the terminal -CH<sub>2</sub>OH group by ENDOR. This situation in the case of the alcohol derivative arises because the rotation around the terminal C-C bond of the side chain is not restricted as it is in the conjugated systems studied here.

The present investigation of the electronic absorption properties of spin-labeled carbonyl derivatives provides much promise for the use of these compounds as spectrophotometric probes in addition to their use as probes for EPR and ENDOR spectroscopy. In the accompanying publication,<sup>27</sup> we will also provide an example to illustrate the use of spin-labeled propenal IV as a kinetically specific and catalytically reactive substrate probe of the enzyme horse liver alcohol dehydrogenase.

(27) Mustafi, D.; Boisvert, W. E.; Mäkinen, M. W. *J. Am. Chem. Soc.*, following paper in this issue.