Synthesis of Mono-, Di-, and Polynitroxides. Classification of Electron Spin Resonance Spectra of Flexible Dinitroxides Dissolved in Liquids and Glasses

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Abstract: The synthesis of flexible biradical strain gauges which could be anchored onto two sites of a deformable biological structure had been attempted. Qualitative relationships between the possible conformations of the biradicals and the observed esr spectra provided guidelines for further synthetic work. Some of the nitroxides synthesized and characterized are $RCONR_1(CH_2)_nNR_2COR$, where R is 1-oxyl-2,2,5,5-tetramethylpyrroline bonded at the 3 position, n = 2,3, and $R_{1,2} = -H$; $-C_{18}H_{37}$; $-CH_2COOH$; $-CH_2COOC_2H_5$; $-CH_2CONH(CH_2)_2N(CH_3)_2$; $-CH_2CONH(CH_2)_2N^+(CH_3)_3I^-$; $-CH_2CO$ imidazolide.

Spin labeling[6-8] is one of the reporter group techniques of molecular biology[9] in which stable free radicals are extrinsically applied to biological specimens which are then studied by esr spectroscopy. The stable free radicals[10] most commonly used for spin labeling are the methyl-protected iminoxyls[6,9-11] ("nitroxides"), in which chemical stability is combined with simplicity of the esr spectrum.

Pairwise spin interactions have been manifest in the esr spectra of *biradicals* by the appearance of new component lines[12,13] and a correspondence has been found between the intensity of the spin interactions and the conformation of flexible biradicals.[12-21]

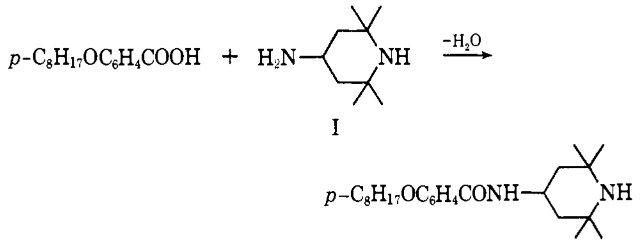
Biophysical applications, in which conformations can be deduced from the features of esr spectra which depend on the intensity of the pairwise spin interactions, have been proposed and demonstrated.[22-24]

The present authors have proposed[24] a flexible biradical *strain gauge* (geometrical probe), which would be attached to a biological sample *at two points*, deform together with the support, and transduce the strain into the interaction-dependent features of the esr spectra.

The preparation and application of the strain gauge biradicals required the preliminary work reported here: (a) the synthesis of several biradicals, each equipped with two functional groups for covalent, noncovalent or ionic bonding to biological samples; (b) modeling of the spectra expected from the biradical strain gauge, and (c) the synthesis of mono-, bi-, and polyradicals not directly related to the strain gauge project.

Nitroxyl Radicals in the Piperidine Series. Stable free radicals derived from suitably substituted 1-oxyl-2,2,6,6-tetramethylpiperidine have been useful in studying molecular ordering in nematic mesophases.[13,16,18,20,25,26] In these studies the long molecular axis of the solute radical is required to coincide with one of the principal axes of the anisotropic spin interactions, Some radicals fitting this description (Table I, IV–VI) have been synthesized.

Selective acylation of the primary amino groups of 2,2,6,6-tetramethyl-4-aminopiperidine (I) with p-octyloxybenzoic acid by the carbodiimide method[27] gave the expected 2,2,6,6-tetramethyl-4-(p-octyloxy)benzoylaminopiperidine[25] (II)



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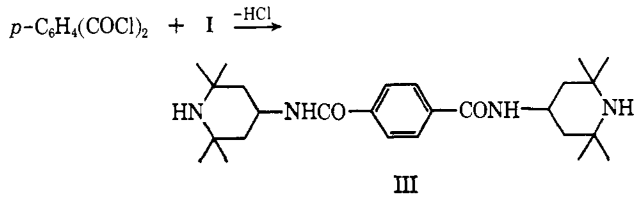
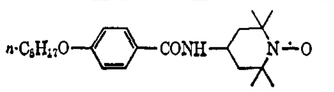
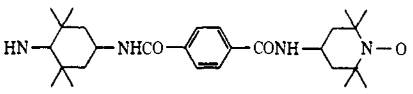
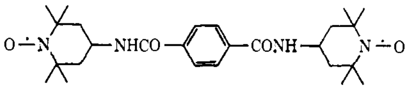
Selective acylation of I with terephthaloyl chloride gave N,N-bis-4-(2,2,6,6-tetramethyl)piperidylterephthalamide (III)



III

The catalytic oxidation[28] of II gave radical IV (Table I), the chemistry of which has been described elsewhere.[25] The catalytic oxidation of III gave a mixture of the radical V and the biradical VI (Table I). These two latter radicals can be separated easily since only V is soluble in acidic aqueous solutions.

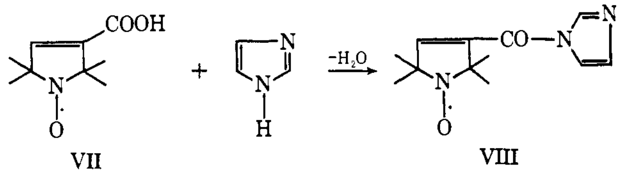
Table I. Piperidine-Derived Mononitroxides (IV, V) and a Dinitroxide (VI)

No.[b]	Name	Structural formula
IV	1-Oxyl-2,2,6,6-tetramethyl-4-(*p*-octyloxy)benzoylaminopiperidine[a]	
V	N-4-(2,2,6,6-Tetramethyl)piperidyl-N′-4-(1-oxyl-2,2,6,6-tetramethyl)piperidylterephthalamide	
VI	N,N′-Bis-4-(1-oxyl-2,2,6,6-tetramethyl)piperidylterephthalamide	

[a] See ref 22. [b] Esr of IV–VI in nematic solvents,[25,26] as well as simulation by space-filling models indicate that the ring assumes an average planar configuration in which the N–O bond direction is nearly parallel to the long molecular axis. Conformational exchange is not possible between the nitroxide subunits of VI because the molecule is rigid. Dipolar interaction, however, is evident in the broadening of the "three-line" spectrum of VI in dilute solutions.

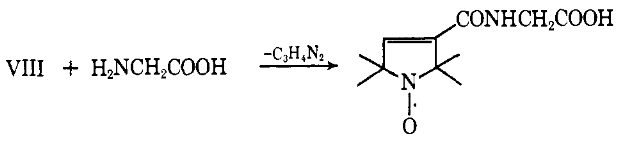
Nitroxyl Radicals in the Hydrogenated Pyrrole Series. We prepared a number of mono-, bi-, and multiradicals deriving from substituted 1-oxyl-2,2,5,5-tetramethylpyrroline.[28-31] These radicals have been used to study nerve membranes.[24]

A. Monoradicals. A radical was obtained by coupling imidazole with 1-oxyl-2,2,5,5-tetramethyl-3-carboxypyrroline (VII), the latter obtained by the procedure of Rozantsev and Krinitzkaya.[26] We used 1-ethyl-3-(3-N,N-dimethylaminopropyl)carbodiimide hydrochloride as the coupling agent. The same product may be obtained from VII and N,N′-carbonyldiimidazole,[32] but with a lower yield.



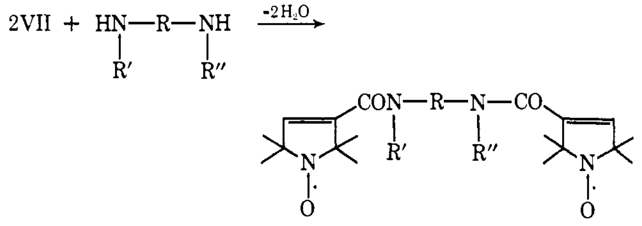
VII VIII

Owing to the general reactivity of the carboxylic acid imidazolides toward primary and secondary amines, alcohols and phenols, radical VIII is a very useful chemical intermediate as well as a potential spin label for biological systems. For example, the condensation of VIII with glycine results in the new radical acid, N-(1-oxyl-2,2,5,5-tetramethylpyrroline-3-carboxy)glycine (IX).



IX

B. Biradicals. Coupling of the acid radical VII with a diamine by the carbodiimide method was found to be suitable for synthesizing a large number of biradicals of varying physical and chemical properties: Biradicals obtained by this method, with R′ and R″ being H or alkyl groups and R being an methylene chain, are listed in Table II. The esr spectra of radicals X, XI, and XIV have been published previously.[24] The esr spectra of radicals XII and XIII show varying degrees of steric interference with collisional exchange similar to those reported in ref 21.



The coupling of VII with the diethyl ester of N,N′-ethylenediamine-N,N′-diaminediacetic acid (XV) enabled us to obtain biradical spin labels with specific chemical functions. Hydrolysis of the resulting diethyl ester of the N,N-bis-(1-oxyl-2,2,5,5-tetramethylpyrroline-3-carboxy)ethylenediamine-N,N′-diacetic acid (XVI) yielded the free acid (XVII). This diacid biradical XVII was, in turn, coupled with 2-dimethylaminoethylamine to give its N,N′-bis(2-dimethylaminoethyl)diamide (XVIII). Treatment of XVIII with methyl iodide finally gave the corresponding bisquaternary ammonium salt (XIX). The aforementioned reactions are summarized in Table III.

The three aforementioned radicals (XVII, XVIII, and XIX) are expected to ionize in aqueous solutions, thus providing charged functional groups. These charged groups are intended to provide coulombic interaction with charged sites of biological structures.

A reactive biradical with two imidazole groups at each end of the molecule was also prepared by reacting one part of the diacid XVII (Table III) with two parts of imidazole in the presence of carbodiimide (using the same procedure as in the synthesis of VIII). The

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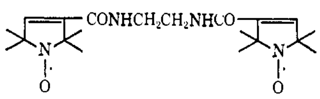
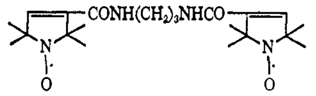
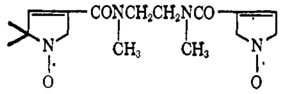
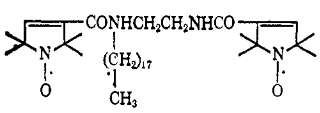
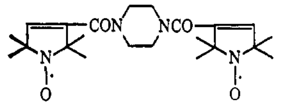
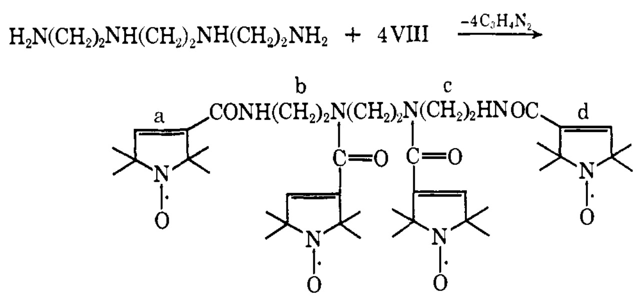
Table II. Lyophylic Pyrroline Derived Dinitroxides

No.	Name	Structural formula
X[a]	N,N′-Bis(1-oxyl-2,2,5,5-tetramethylpyrroline-3-carboxy)-1,2-diaminoethane	
XI[a]	N,N′-Bis(1-oxyl-2,2,5,5-tetramethylpyrroline-3-carboxy)-1,3-diaminoethane	
XII	N,N′-Dimethyl-N,N′-bis(1-oxyl-2,2,5,5-tetramethylpyrroline-3-carboxy)-1,2-diaminoethane	
XIII[a]	N-*n*-Octadecyl-N,N′-bis(1-oxyl-2,2,5,5-tetramethylpyrroline-3-carboxy)-1,2-diaminoethane	
XIV	1,4-Bis(1-oxyl-2,2,5,5-tetramethyl-3-carboxy)piperazine	

[a] Comparative measurements of the direct exchange in solutions of X and XI indicate that the interaction weakens with the lengthening of the chain which separates the radical subunits. XIII has a long aliphatic chain for attachment to lipid matrices.

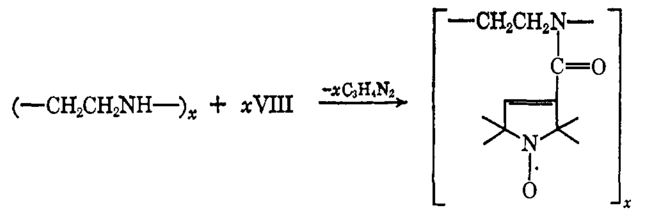
diimidazole **XX** of **XVII** was obtained as a sticky yellowish oil.

C. **Polynitroxides.** A tetranitroxide has been constructed by condensing the radical acid VII with triethylenetetramine by using the carbodiimide method. The product was N,N′,N′′,N′′′-tetra(1-oxyl-2,2,5,5-tetramethylpyrroline - 3 - carboxyl)triethylenetetramine (XXI). The esr spectrum of the tetraradical is shown in Figure 9.



XXI

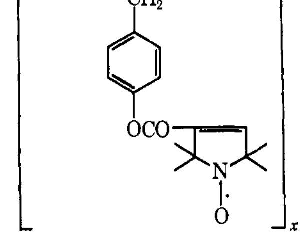
The radical imidazolide (VIII) was used as an acylating reagent to obtain *radical polymers.* In fact, treatment of suitable macromolecular compounds containing acylatable functions with VIII in excess results in almost complete acylation without side reactions. The resulting radical polymer is easily purified from imidazole and the excess of VIII, as well as from radical acid (VII) which may be formed as a side product by the hydrolysis of a part of VIII when the reaction is performed in an aqueous medium.



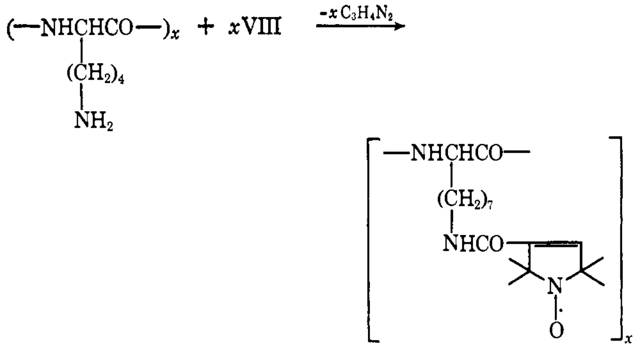
XXII

By the above method, we have prepared poly-N-(1-oxyl-2,2,5,5-tetramethylpyrroline-3-carboxy)ethylenimine (XXII).

We have also prepared poly-O-(1-oxyl-2,2,5,5-tetramethylpyrroline - 3 - carboxy) - L - tyrosine (XXIII)

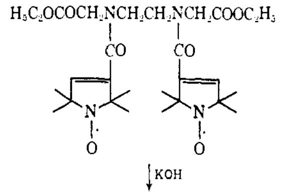
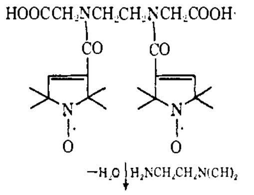
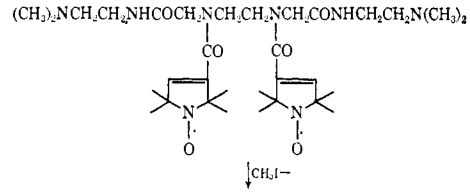
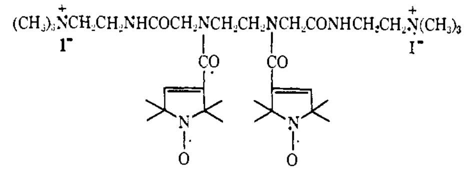
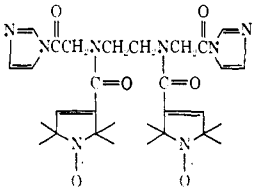


XXIII



XXIV

Table III. Pyrroline-Derived Dinitroxides[a,b]

No.	Structural formula
XVI	
XVII	
XVIII	
XIX	
XX	

[a] The pyrroline-derived dinitroxides are equipped with (a) two ionizable groups (XVII–XIX) which would bond ionically to charged sites on macromolecules, and (b) two imidazolide groups (XX) which bond convalently to side chains of proteins. [b] Biradical XVII is the starting material for the synthesis. Biradicals XVIII–XIX in aqueous solutions have displayed an interesting pH dependence of the direct exchange interaction. Ionization introduces a repulsive electrostatic force between the ionization groups which brings the nitroxide groups closer than in the neutral molecule, a situation described in entry D of Table IV.

and poly-N-(1-oxyl-2,2,5,5-tetramethylpyrroline-3-carboxyl)-L-lysine (XXIV).

Experimental Section

N,N-Bis-4-(2,2,6,6-tetramethyl)piperidylterephthalamide (III). A solution of 2.03 g of terephthaloyl chloride in 40 ml of chloroform was added dropwise into a stirred solution of 3.12 g of I in 35 ml of chloroform, cooled at ice temperature. The reaction mixture was stirred 1 hr while warming at room temperature, and then refluxed for a few minutes. After cooling, the white dihydrochloride of III was filtered and dissolved in 300 ml of aqueous 0.5 *N* hydrochloric acid. The base was then precipitated with excess of sodium hydroxide, filtered, and recrystallized from isopropyl alcohol: mp 308–310° dec; yield 3.0 g (68%). *Anal.* Calcd for $C_{26}H_{42}N_4O_2$: N, 12.77. Found: N, 12.66.

N-4-(1-Oxyl-2,2,6,6-tetramethyl)piperidyl-N′-4-(2,2,6,6-tetramethyl)piperidylterephthalamide (V) and N,N′-Bis-4-(1-oxyl-2,2,6,6-tetramethyl)piperidylterephthalamide (VI). A 1-ml sample of a 30% hydrogen peroxide solution was added to a stirred suspension of 0.25 g of I, 0.05 g of EDTA, and 0.05 g of sodium tungstate in 10 ml of methanol. After stirring at room temperature for 7 days, the mixture was poured into 150 ml of 0.5 *N* aqueous hydrochloric acid. The resulting suspension was then extracted 15 times with 40-ml portions of chloroform.

The aqueous phase was filtered and made alkaline with sodium hydroxide. The resulting precipitate was recovered by filtration and dried to give 0.03 g (11.5%) of V, which was then recrystallized from methanol: mp 298–301° dec. *Anal.* Calcd for $C_{26}H_{41}N_4O_3$: C, 67.76; H, 9.19; N, 11.94. Found: C, 68.23; H, 9 03; N, 12.24).

The organic phase was washed with diluted hydrochloric acid, water, and saturated sodium chloride solutions before drying over anhydrous sodium sulfate. The solvent was then removed *in vacuo* to give 0.21 g (78.5%) of VI, which was recrystallized with some losses from isopropyl alcohol: mp 274–276° dec. *Anal.* Calcd for $C_{26}H_{40}N_4O_4$: C, 66.22; H, 8.28; N, 11.90. Found: C, 66.07; H, 8.53; N, 11.86.

1-(1-Oxyl-2,2,5,5-tetramethylpyrroline-3-carboxy)imidazole (VIII). A solution of 0.92 g of VII, 1.15 g of 1-ethyl-3-(3-N,N-dimethylaminopropyl)carbodiimide hydrochloride (hereafter referred to as carbodiimide) and 0.34 g of imidazole in 30 ml of chloroform was stirred at ice temperature for 1 hr, followed by 8 hr of stirring while warming to room temperature. The yellow mixture was filtered and extracted twice with 50-ml portions of water, then dried over anhydrous sodium sulfate. After filtration to remove the drying agent, the solution was concentrated *in vacuo* to yield 1.10 g (94%) of yellow crystalline solid, which was recrystallized from *n*-hexane: yellow needles; mp 101–102°. *Anal.* Calcd for $C_{12}H_{16}N_3O_2$: C, 61.36; H, 7.16; N, 17.98. Found: C, 61.52; H, 6.88; N, 17.94.

By adding VII at room temperature to a slight excess of 1,1′-carbonyldiimidazole in chloroform, stirring at room temperature for 30 min, and then working up the solution as previously described, VIII is obtained in 75% yield.

N-(1-Oxyl-2,2,5,5-tetramethylpyrroline-3-carboxy)glycine (IX). A mixture of 0.300 g of glycine, 1.6 ml of aqueous 10% NaOH, and ≃1 g of ice was treated with 0.20 g of VIII. The mixture was vigorously stirred while warming at room temperature until a clear solution was obtained, then left at room temperature for 1 hr. The solution was then acidified with hydrochloric acid and extracted three times with chloroform. After drying with anhydrous sodium sulfate, the solution was concentrated *in vacuo* to yield 0.085 g (40% based on VIII) of yellow oil which slowly crystallized by rubbing under cold *n*-hexane. It was recrystallized by dissolving in the minimum amount of boiling ethyl acetate–*n*-hexane 1:2 (v/v), then adding two more volumes of *n*-hexane and keeping for awhile at room temperature: yellow powder; mp 130–131°. *Anal.* Calcd for $C_{11}H_{17}N_2O_4$: C, 55.09, H, 7.21; N, 11.26. Found: C, 54.76; H, 7.10; N, 11.61.

N,N′-Bis(1-oxyl-2,2,5,5-tetramethylpyrroline-3-carboxy)-1,2-diaminomethane (X). A mixture of 1.84 g of VII, 2.30 g of carbodiimide, and 0.30 g of ethylenediamine in 30 ml of chloroform was stirred at ice temperature for 2 hr, followed by 8 hr of stirring while warming at room temperature. The yellow reaction mixture was filtered and washed with water, 2 *N* sulfuric acid, water, and 5% sodium chloride solution before drying over anhydrous sodium sulfate. After removing the drying agent, the filtrate was concentrated *in vacuo* to give 1.41 g (72%) of X, which may be recrystallized from chloroform–ethyl ether: pale yellow needles; mp 226–227° dec. *Anal.* Calcd for $C_{20}H_{32}N_4O_4$: C, 60.90; H, 8.43; N, 14.17. Found: C, 61.17; H, 8.23; N, 14.27.

N,N′-Bis(1-oxyl-2,2,5,5-tetramethylpyrroline-3-carboxy)-1,3-diaminopropane (XI). A mixture of 0.92 g of VII, 1.15 g of carbodiimide, and 0.185 g of 1,3-propylenediamine in 15 ml of chloroform was processed as previously described. The product was recrystallized from benzene: yellow powder; mp 180° dec; yield 0.5 g (49%). *Anal.* Calcd for $C_{21}H_{34}N_4O_4$: N, 13.64. Found: N, 13.79.

N,N′-Dimethyl-N,N′-bis(1-oxyl-2,2,5,5-tetramethylpyrroline-3-carboxy)-1,2-diaminoethane (XII). A mixture of 0.46 g of VII, 0.57 g of carbodiimide, and 0.11 g of 1,2-bis(methylamino)ethane in 10 ml of chloroform was processed as previously described. The product was recrystallized by dissolving in warm chloroform and diluting with excess ether: pale yellow powder; mp 260° dec, yield 0.31 g (61.5%). *Anal.* Calcd for $C_{22}H_{36}N_4O_4$: C, 62.35; H, 8.45; N, 12.97. Found: C, 62.83; H, 8.63; N, 13.32.

N-*n*-Octadecyl-N,N′-bis(1-oxyl-2,2,5,5-tetramethylpyrroline-3-carboxy)-1,2-diaminoethane (XIII). A mixture of 0.92 g of VII, 1.15 g of carbodiimide, and 0.78 g of N-*n*-octadecyl-1,2-diaminoethene[33] in 30 ml of chloroform and 30 ml of pyridine was stirred

Table IV. Qualitative Correlation of Observed Esr Spectra of Flexible Dinitroxides with the Conformations and Tumbling Rates in Various Solvents[a]

		Frozen conformations		
			Bent	
	Thermally activated conformations	Straight	No exchange	Exchange
	A	B	C	D
Free tumbling	"Five-line" spectrum. Spin exchange by thermally activated conformational collisions of the subunits. (Figure 6, 180°)	"Three-line" spectrum. No exchange because of conformational hindrance. Transforms into F upon gradual freezing. (Figure 1, 40°)	"Three-line" spectrum. Transforms into G upon gradual freezing.	"Five-line" spectrum. Exchange is assisted by frozen, bent conformation and weakens when straight conformations are activated. (Figure 4, pH 10.8).
	E	F	G	H
Hindered tumbling	The ideal doubly anchored biradical strain gauge (geometrical probe).	Powder pattern spectrum identical with that of diluted mononitroxide frozen in a glass. (Figure 3, 24°)	Powder superposition of spectra, the hyperfine components which are split by dipolar interaction. (Figure 5, 20°)	The spectrum consisting of one broad line with two weak and broad satellites is contributed by all the interactions present in biradicals. (Figure 6, 24°, Figure 7)

[a] The rows characterize the tumbling motion of the biradical molecules as either free or frozen. The columns grossly specify the conformation as straight (large r) or bent (small r). A further subdivison relates to the internal degrees of freedom which facilitate direct spin exchange by the overlap of the singly occupied orbitals in the bent biradical.

for 1 hr at ice temperature, followed by 24 hr of stirring while warming to room temperature. The solution was then diluted further with 30 ml of chloroform, filtered, and washed twice with water, twice with 2 *N* acetic acid, with water, with cold 0.5 *N* sodium hydroxide, with water, and with saturated sodium chloride solution before drying over anhydrous sodium sulfate. After evaporating the solvent *in vacuo* the crude product was a yellow oil. The oil was dissolved in warm *n*-hexane and left at room temperature for 2 hr. A crystalline solid, homogeneous by tlc (SiO_2–$CHCl_3$), precipitated out and was recrystallized from *n*-hexane to give 0.35 g (21.7%) of XIII: pale yellow powder; mp 84–85°. *Anal.* Calcd for $C_{38}H_{68}N_4O_4$: C, 70.45; H, 10.71; N, 8.77. Found: C, 70.77; H, 10.63; N, 8.69.

1,4-Bis(1-oxyl-2,2,5,5-tetramethylpyrroline-3-carboxy)piperazine (XIV). A mixture of 1.67 g of VII, 2.10 g of carbodiimide, and 0.39 g of freshly recrystallized piperazine in 30 ml of chloroform was processed as described in the preparation of X. The crystalline product (1.45 g, 76.5%) was recrystallized by dissolving in warm chloroform and diluting with an excess of ether: yellow needles; mp 260° dec. *Anal.* Calcd for $C_{22}H_{34}N_4O_4$: C, 63.16; H, 8.13; N, 13.39. Found: C, 62.94; H, 8.13; N, 13.55.

Diethyl Ester of Ethylenediamine-N,N′-diacetic Acid (XV). A suspension of 5 g of ethylenediamine-N,N′-diacetic acid (purchased from K & K) in 350 ml of absolute ethyl alcohol was saturated with gaseous hydrogen chloride and refluxed for 10 hr. An almost clear solution was obtained. The solution was filtered while hot and then chilled in an ice bath for 1 hr. The hydrochloride of XV crystallized out as white leaflets and was recrystallized from absolute ethyl alcohol: mp 170° dec; yield 4.4 g (50%). *Anal.* Calcd for $C_{10}H_{22}N_2O_4Cl_2$: N, 9.00. Found: N, 9.18.

The above hydrochloride was treated in chloroform with triethylamine; the solution was filtered and poured into excess diethyl ether. After filtration of the resulting triethylamine hydrochloride, by evaporating the solvents *in vacuo*, compound XVI was obtained as a clear oil. This was used in the subsequent step without further purification.

Diethyl Ester of N,N′-Bis(1-oxyl-2,2,5,5-tetramethylpyrroline-3-carboxy)ethylenediamine-N,N′-diacetic Acid (XVI). A mixture of 1.38 g of VII, 0.87 g of XVI, and 1.75 g of carbodiimide in 20 ml cf $CHCl_3$ was stirred 1 hr at ice temperature, followed by stirring 8 hr at room temperature. The resulting clear solution was washed with water, 2 *N* sulfuric acid, water, 5% sodium bicarbonate solution, water, and saturated sodium chloride solution. After drying over anhydrous sodium sulfate and filtering, the solution was evaporated to dryness *in vacuo* to yield 1.15 g (54%) of XVI as an orange-red oil, homogeneous by tlc (SiO_2–$CHCl_3$) which was directly hydrolyzed to XVII without further purification.

N,N′-Bis(1-oxyl-2,2,5,5-tetramethylpyrroline-3-carboxy)ethylenediamine-N,N′-diacetic Acid (XVII). A mixture of 0.72 g of XVI and 2.8 ml of methanolic 1 *N* potassium hydroxide was stirred 2 hr at 40°; water (5 ml) was added and the mixture was refluxed 1 min. After cooling and adding more water (15 ml), the mixture was extracted twice with ether, and the ethereal extracts were discarded. The resulting clear aqueous solution was acidified with hydrochloric acid and extracted twice with chloroform. The combined chloroform extracts were washed with saturated sodium chloride solution and dried over anhydrous sodium sulfate. After filtration to remove the drying agent the filtrate was evaporated to dryness *in vacuo*. The residue was dissolved in 25 ml of chloroform–methanol 10:1 (v/v) and the solution poured in 400 ml of ether–petroleum ether (bp 30–60°) 1:10 (v/v) to yield 0.24 g (36.3%) of pure XVIII: pale yellow powder; mp 250° dec. *Anal.* Calcd for $C_{24}H_{36}N_4O_8$: C, 56.49; H, 7.11; N, 11.29. Found: C, 56.68; H, 7.14; N, 11.02.

N,N′-Bis(2-dimethylamino)ethyldiamide of XVIII. A mixture of 0.152 g of XVII, 0.052 g of 2-dimethylaminoethylamine, and 0.150 g of carbodiimide in 3 ml of chloroform was stirred 1 hr at ice temperature followed by 10 hr of stirring at room temperature. The resulting clear solution was diluted with 15 ml of chloroform, washed with water, 0.1 *N* sodium hydroxide, water, and saturated sodium chloride before drying over anhydrous sodium sulfate. The drying agent was removed by filtration and the solution was evaporated to dryness *in vacuo* to give a yellow oil which partly crystallized by rubbing under *n*-hexane. The product was dissolved in ether (about 5 ml) and poured in a large excess of *n*-hexane chilled at 0°. This procedure was repeated twice to yield 0.1 g (42.6%) of XVIII which softens at 63–65° and melts completely at 80–82°. *Anal.* Calcd for $C_{32}H_{56}N_8O_6$: N, 16.93. Found: N, 17.27.

Dimethiodide of XVIII, XIX. A solution of 0.08 g of XVIII in 1 ml of chloroform was treated with 0.1 ml of methyl iodide and left at room temperature 1 hr with occasional stirring. A precipitate was formed. The suspension was diluted with 2 ml of ether, and the crystalline XIX filtered and washed thoroughly with ether: yield 0.110 g (95.6%). The product begins to decompose at about 140° and melts completely with decomposition at 205–210°. *Anal.* Calcd for $C_{34}H_{62}N_8O_6I_2$: N, 11.77. Found: N, 12.01.

N,N′,N′′,N′′′-Tetra(1-oxyl-2,2,5,5-tetramethylpyrroline-3-carboxy)triethylenetetramine (XXI). A mixture of 0.097 g of triethylenetetramine, 0.491 g of VII, and 0.65 g of carbodiimide in 10 ml of chloroform was processed as previously described in the preparation of the biradical X: yield 0.08 g (15.2%) of a crystalline product which may be recrystallized from benzene; mp 216–217°. *Anal.* Calcd for $C_{42}H_{66}N_8O_8$: N, 13.98. Found: N, 13.82.

Poly-N-(1-oxyl-2,2,5,5-tetramethylpyrroline-3-carboxy)ethyleneimine (XXII). Polyethylenimine (0.06 g) of M_n 10,000 was dissolved in 10 ml of a dioxane–water mixture, containing the minimum amount of water necessary to keep the polymer in solution, and 0.350 g of VIII was added. The solution was stirred 2 weeks at room temperature. The reaction mixture was then evaporated to dryness *in vacuo*, the residue was dissolved in absolute methanol and poured into excess of ether–petroleum ether 1:2 (v/v). The precipitated XXII was thoroughly washed with ether and dried at

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(33) F. Linsker and R. L. Evans, *J. Amer. Chem. Soc.*, **68**, 1432 (1946).

room temperature under vacuum: yield 0.325 g (80.6%). *Anal.* Calcd for $[C_{11}H_{17}N_2O_2]_x$: N, 13.59. Found: N, 13.39. The analysis suggests that the degree of acylation is practically quantitative (the nitrogen content of polyethylenimine is 32.52%).

Poly-O-(1-oxyl-2,2,5,5-tetramethylpyrroline-3-carboxy)-L-tyrosine (XXIII). Poly-L-tyrosine (0.150 g) of M_n 100,000 was dissolved in 5 ml of anhydrous dimethylformamide and 0.3 g of VIII was added. After stirring 48 hr at room temperature the reaction mixture was poured into 50 ml of ether. The precipitated XXIII was dissolved again in dimethylformamide and reprecipitated with excess ether, thoroughly washed with ether, and dried at room temperature under vacuum: yield 0.095 g (97.4%). The product neither dissolved nor swelled in aqueous sodium hydroxide. This indicates that the acylation was practically quantitative.

Poly-N-(1-oxyl-2,2,5,5-tetramethylpyrroline-3-carboxy)-L-lysine (XXIV). Poly-L-lysine hydrobromide (0.035 g) of M_n 100,000 was dissolved in 2 ml of water, and the solution was made alkaline with 0.1 ml of triethylamine. While stirring vigorously, 0.1 g of solid VII was added; after a few seconds a precipitate began to appear. Dioxane (4 ml) was then added in order to have a homogeneous mixture; a further quantity (0.03 g) of VIII was also added and the resulting clear solution was stirred at room temperature for 12 hr. The reaction mixture was then poured into a large volume of water; the precipitated XXIII was thoroughly washed with water, dissolved in dimethylformamide, and reprecipitated in ether. After washing several times with ether, the polymer was dried at room temperature under vacuum, yield 0.068 g (84.5%). The product neither dissolved nor swelled in aqueous hydrochloric acid. This indicates a practically quantitative degree of acylation.

Esr Spectra

The spectra described here have been taken at *x* band in a Varian E-3 spectrometer.

Mononitroxides. The esr spectra of isolated methyl-protected nitroxides with a particular orientation with respect to the magnetic field exhibit three component lines separated by the anisotropic hyperfine interaction between the electron and nuclear spin of nitrogen and centered at a field value determined by the anisotropic *g* tensor.[6–11] When the radicals are frozen in a glass, the esr spectrum assumes a broad "powder pattern."[34] Molecular motion narrows this spectrum which, in the limit of rapid isotropic tumbling, consists of three sharp lines separated by the isotropic average of the ^{14}N hyperfine tensor and centered at a field value determined by the isotropic average of the *g* tensor.[34]

Dinitroxides. Each of the dinitroxides described here contains two radical groups connected by a flexible saturated hydrocarbon chain (Tables I–III). The electron spins of the nitroxide subunits may interact in a way which markedly modifies the esr spectra; these interactions depend on the distance and angle between the subunits and thus the spectral modifications may be interpreted in terms of the conformation of the biradical.[13,22,24]

This proposition is complicated by the fact that the two known types of pairwise spin interactions depend not only on the radical separation distance, *d*, between the subunits but also on external or on internal coordinates not obviously related to *d*.

The dependence on distance as well as on *external angular coordinates* is evident in the dipolar interaction which is effective in splitting each hyperfine component of the esr spectrum when the tumbling motion of the biradical is arrested. With this condition fulfilled, the effective range of dipolar interactions measurable by esr can be as large as 17 Å.[13,18] When the molecule tumbles isotropically, the interaction does not split the hyperfine components of the spectrum but does contribute to their widths.

The intramolecular Heisenberg *spin exchange interaction* is brought about by the direct electronic overlap of the free radical subunits, due to conformational collisions,[12,14,15,17] or indirectly by the distribution of unpaired electron spin density through the backbone of the biradical molecule. Indirect exchange usually prevails in biradicals in which the number of bonds separating the subunits is small and/or when these bonds are conjugated.[11] This latter type of exchange is negligibly small in the biradicals reported here.

Direct exchange occurs when the free radical subgroups physically approach to within a range of 6 Å or less.[21] The conformations which a flexible dinitroxide must assume in order to achieve intramolecular direct exchange occur by bending and, in addition, by twisting until the singly occupied $N_{p\pi}$ orbitals of the nitroxide subunits are nearly collinear.[23] The scalar exchange interaction does not depend on external angular coordinates and is fully effective in rapidly tumbling molecules. On the other hand, internal angular coordinates not uniquely related to the radical separation distance *d* are involved in the process. Inspection of space-filling CPK molecular models, as well as the observation of esr spectra, convinced us that neither one of the very flexible biradicals **X–XX** would be a good strain gauge, because the distance between the free radical subunits does not uniquely represent the distance between two anchoring groups. As a result of the freedom to twist about the $-CH_2CH_2-$bonds, the models indicate that the radical subunits are likely to be in "*trans*" conformations (observed in models).

In spite of these shortcomings, biradicals **X–XX** provided partial answers to the following questions. (1) Can the merit of the doubly anchored biradical strain gauge be inferred from the appearance of pairwise interactions in the esr spectra in solution? (2) Which of the two pairwise interactions, dipolar or exchange, would be more useful as a conformational indicator in a doubly anchored strain gauge? (3) Are the esr spectra of *frozen* biradicals representative of the spectra of the same biradicals when double anchored to a flexible support?

The common feature of most of the biradicals (X, XII–XIII, XVI–XX) is the $-CONH(CH_2)_2NHCO-$ connecting chain. This chain was found to permit conformational spin exchange *in solution*. Later the biradicals were applied to deformable supports such as rubber (X and XIII), ion-exchange resins (XVII–XIX), polypeptides (XX), and biological samples. Esr spectra were taken while the supports were deformed. The spectra did *not* indicate any feature clearly ascribable to pairwise interactions and did not transduce the state of strain of the support. Thus, the performance of the anchored strain gauge *cannot* be inferred from its spectra in solution.

The line widths of the biradical spectra in *solution* were *broader* than those of the corresponding monoradicals and were sometimes *reduced* in the *anchored* biradicals. We ascribed the broadening in solution to electron–electron dipolar fluctuations which subsided when the biradicals were anchored. Strong immobilization was then brought about by freezing and the accompanying broadening was ascribed to other than thermal relaxation process interactions.

The absence of exchange in the anchored biradicals

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(34) M. S. Itzkowitz, *J. Chem. Phys.*, 46, 3048 (1967).

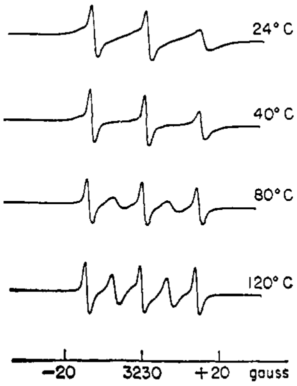

Figure 1. First derivative esr spectra of biradical X in *n*-octyl alcohol. The spectra at 24 and 40° typically represent case **B** of Table IV, in which the tumbling of the biradicals is rather free, while the conformation is straight and still frozen. The straightening effect is typical of alcohol solvents. The pure "three line" appearance of the spectrum at 40° indicates that indirect exchange through the backbone of the biradical molecule is negligible. The changes in the spectra at 80 and 120° are due to direct exchange brought about by the thermally activated bending of the biradical. The addition of water loosens the conformational stiffness in the alcohol, and lowers the temperature at which exchange effects appear in the spectra.

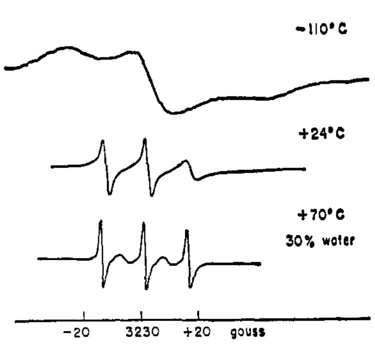

Figure 2. First derivative esr spectra of biradical XVII in ethylene glycol. (a) The spectrum at −120° represents case F in Table IV because it is indistinguishable from that of noninteracting mononitroxides frozen in a glass. (b) AB type of spectrum at room temperature. (c) Exchange was observed only after the addition of up to ~30% water and heating to 70°. The straightening of the biradicals by the $(CH_2OH)_2$ solvent has been so firm that only the addition of ~30% water and heating to 70° could produce the exchange effects in this figure.

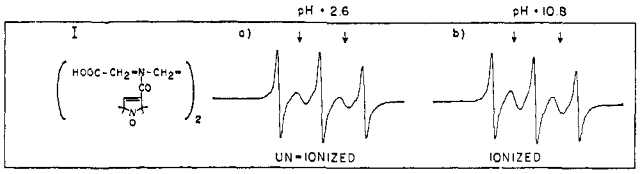

Figure 3. First derivative esr spectra of XVII in water solutions at pH 2.6 (left) and pH 10.8 (right). At acidic pH (left) the carboxyl groups are neutral and the biradical assumes conformations in which the carboxyls are close and the nitroxide subunits are separated. In the basic environment (right), the carboxyls ionize and repel each other so that in the resulting conformations the nitroxide subunits are close. Direct exchange is thus more likely. This is a striking illustration of case D in Table IV.

indicates that nothing short of direct collision of the $\dot{N}O$ subunits would permit this interaction. Dipolar interaction was not observed, probably because of the residual mobility of the biradicals. An additional difficulty is that conformational inferences from observed biradical spectra can be vitiated by partial chemical destruction which would leave a surviving population rich in monoradicals. This phenomenon was encountered in pure formic acid, in heated glycerol, and in numerous biological samples. Intensity measurements would, of course, identify chemical destruction.

Gradual freezing of the sample gave rise to three types of biradical spectra: (a) those showing no pairwise interaction, (b) dipolar-split ones, and (c) spectra of biradicals in which both interactions are operative. We chose to summarize the conformational significance of the observed spectra in Table IV, in which the rows are descriptive of the external angular degrees of freedom (tumbling) and the columns enumerate the conformational (internal) degrees of freedom. The arrangement of columns is complicated because one first has to distinguish between large-*d* and small-*d* conformations and then the small-*d* conformations must be subdivided into those which are, and those which are not, favorable to spin exchange. All the degrees of freedom are coarsely classified as free or frozen, depending on whether or not they can be activated at the temperature under which the experiment is run.

Table IV is subdivided into the eight following entries (A–H).

A. All the degrees of freedom are fully activated and the biradicals undergo rapid and unhindered tumbling and bending through conformations, many of which are favorable to spin exchange. Spin-exchanging encounters of the nitroxide subunits are frequent, and the spectrum assumes the "five-line" appearance reported by numerous authors[14–21,24] (Figure 6, lowest spectrum).

B. The biradicals tumble freely but their internal degrees of freedom are frozen in a stiff conformation of maximum *d*, in which exchange does not occur and the dipolar interaction is minimal. The spectra consist of three lines only and are identical with those of rapidly tumbling noninteracting mononitroxides. Type B spectra have been observed in three cases: (a) in solutions of rigid molecules in which the nitroxides are separated by a ring structure;[16,24] (b) in solutions of flexible biradicals, the bending motion of which is hindered at low temperatures;[13] (c) the solutions of flexible biradicals which are stiffened by a specific interaction with the solvent[14,16] (Figures 1, 2). Gradual freezing would transform the B spectrum into an F spectrum.

C. The biradicals tumble freely while being constrained in a bent (short *d*) conformation in which, however, exchange is weak because the radical subunits are not overlapping. The only way to distinguish the C spectra from the B spectra is by gradual freezing, as a result of which B passes into F while C passes into G (Table IV). We do not present a typical C spectrum.

D. The biradicals tumble freely while their inner degrees of freedom are frozen in a short-*d* conformation favorable to exchange. The spectrum is indistinguishable from that of case A, yet it may differ from A in its temperature dependence. The bent biradicals would straighten by thermal activation and their exchange would then decrease, or at least not increase at the same rate as in case A. We have been able to demonstrate spectra which fit the description of D.[21]

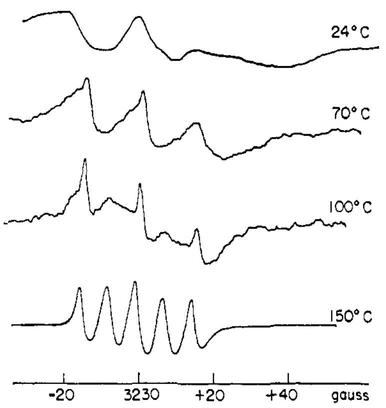

Figure 4. First derivative esr spectra of biradical X in glycerol. The barrier which hinders tumbling is lower than that which hinders bending, in analogy to solutions of other alcohols. The spectrum at 24° fits the description of F in Table IV, the 70–100° spectra approximate case B, and the 150° spectrum is of A type. Heated glycerol chemically reduces the nitroxide radicals.

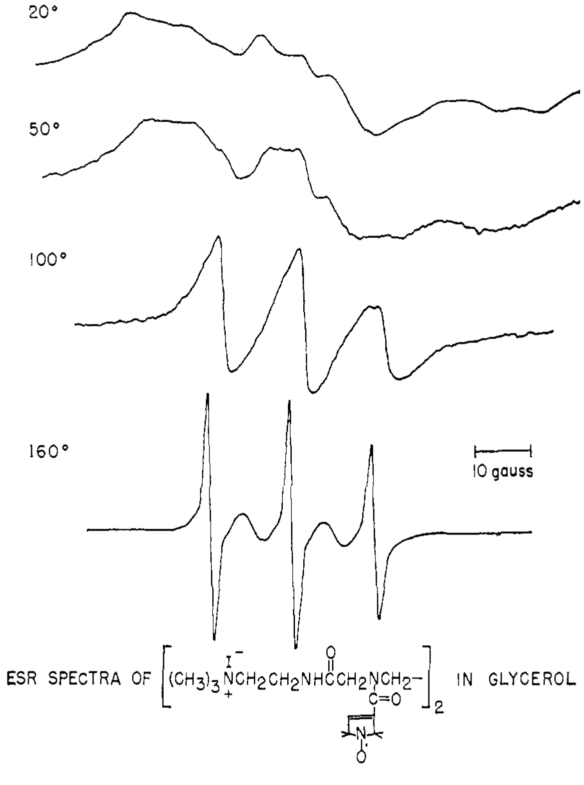

Figure 5. First derivative esr spectra of XIX in glycerol. The spectrum at 20° is representative of case G in Table IV—i.e., the powder pattern spectrum of dinitroxides in which *intramolecular dipolar interaction* is operative. The absence of exchange in the low-temperature spectra is evident because this interaction begins to be activated only at temperatures above 100°. The separation distance R between the nitroxide subunits can be extracted from the 20° spectra by comparison with computer-generated spectra.

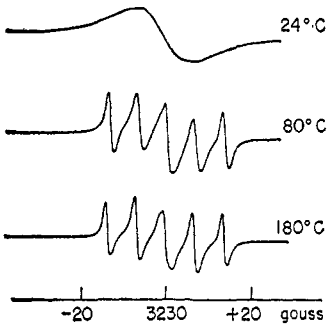

Figure 6. First derivative esr spectra of biradical X in paraffin wax. The spectrum at 24° represents case H in Table IV, in which all the spin interactions, including exchange, are present. The effectiveness of exchange is evident in the straightforward transition of the H spectrum into a type A spectrum upon melting (80°).

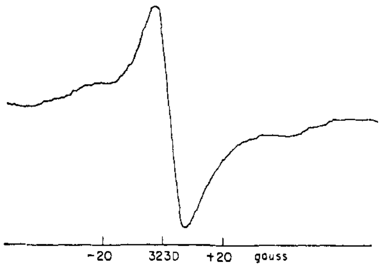

Figure 7. First derivative esr spectrum of biradical X in frozen paraffin wax at room temperature, taken at a large (±5 G) modulation amplitude. Under these conditions the absorption at the wings is discerned. In this powder pattern spectrum all the spin interactions, including exchange, are present.

These are encountered in biradicals XVII and XVIII, each of which has two ionizable groups in addition to the free radical subunits. The polar groups can be ionized by dissolving biradical XVII in a base or XVIII in an acid. The electrostatic repulsion of the ionized groups constrains the rapidly tumbling biradicals into short-*d* conformations in which exchange is enhanced (Figure 3, right).

E. This entry defines the attributes of the desirable biradical strain gauge (geometrical probe), the tumbling motion of which is arrested by anchoring, at two points, to a deformable support while the internal degrees of freedom are unhindered so that the flexions of the biradical follow those of the support. Either one of the pairwise interactions or the combination thereof would transduce the *d* value of the biradical. A biradical meeting these specifications is still awaited.

F. **The Frozen Counterpart of B**. The biradicals are frozen in a stiff conformation with a maximum distance between the radicals. If the separation distance is larger than ~18 Å, the spectra are similar to those of noninteracting mononitroxides frozen in a glass. Spectra of flexible biradicals, stiffened by solvent interaction and frozen, are shown in Figure 2, top, and Figure 4, top.

G. The frozen counterpart of B or C. The only effective pairwise interaction is the dipolar one, which splits each of the hyperfine lines into two components superimposed on a powder pattern spectrum. Figure 5, top, shows a representative spectrum, from which *d* could be derived by comparison with computer simulated spectra.

H. The biradicals are bent and frozen and the two types of pairwise interactions are present. The esr spectrum consists of a broad line (Figure 6, top). By broadly modulating the resonance of X in frozen paraffin wax, we have observed two unresolved, broad wings (Figure 7). We were unable to observe the $\Delta m = 2$ spin triplet transition at one-half the magnetic field at 85°K.

The H-type spectrum, in which the hyperfine, *g* tensor, dipolar and exchange interactions contribute to

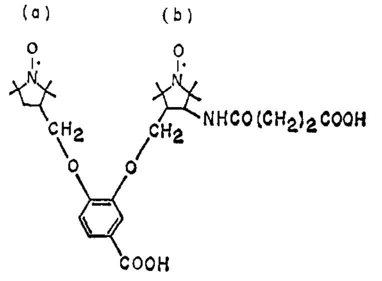

Figure 8. A proposed structure of a biradical strain gauge. Subunit (a) rattles inside a cone and collides with (b) if the two carboxyl groups are relaxed. When the carboxyls are pulled apart, (b) is no more accessible to collision with (a). The carboxyls can be extended into the anchoring groups outlined in Tables II and III.

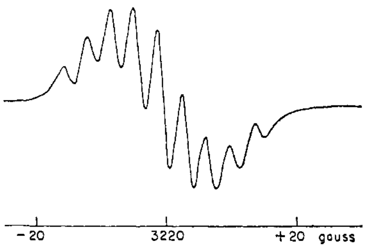

Figure 9. First derivative esr spectrum of tetraradical (XXI) dissolved in 1,2-propylene glycol at 180°. The nine equally spaced components indicate that because of the exchange interaction the four unpaired electrons interact equally with four ^{14}N nuclei. The equality of interactions is also observed at room temperature, but the spectral components are broadened by the viscosity of the solvent.

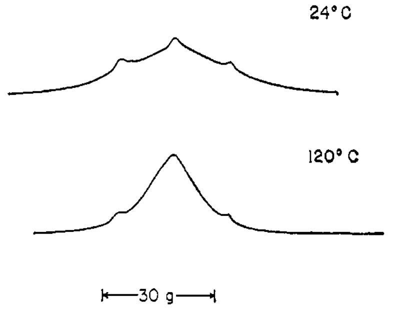

Figure 10. Integrated first derivative esr absorption spectra of completely labeled polytyrosine (XXIII) in solution in DMF at 24° and at 120°. In this solvent the polymer is expected to be a rodlike α-helix in which the spin exchange interaction is limited only to nearest and next-nearest neighbors. The broad peak at 120° can be ascribed to the fraction in the random coil conformation.

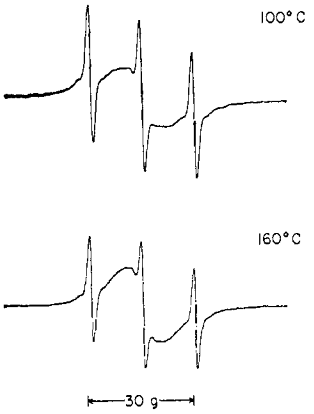

Figure 11. First derivative esr spectra of the labeled polytyrosine in DMF at 100 and 160°, which suggest a superposition of α-helix and random coil contributions.

the powder pattern, can be interpreted only by computer simulation.

We conclude with a proposal for the structure of a biradical molecule which, as far as molecular models indicate, could be a workable strain gauge (Figure 8). The proposal draws from the experience of Hsia[23] and Piette, Rassat and Rey,[35] Kosman and Piette,[36] and ourselves. The weakly immobilized free radical subunit (Figure 8a) bounces freely in the solid angle accessible to it. Subunit b[35] as well as the phenyl ring would be anchored to a biological sample by means of anchoring groups extended from the two free carboxyls. With no strain applied to the carboxyls, the probability of spin exchanging collisions is high. This probability decreases markedly when the carboxyls are pulled apart.

Spectra of Polynitroxides. The synthesis and spectra of several tetraradicals have been reported in the literature.[37,38] In the cases reported the intensity of exchange was equal for all the nitrogen nuclei bonded tetrahedrally in symmetrical tetraradicals. In tetraradicals of lower symmetry, the quoted authors discerned two different intensities of exchange.

We have dissolved tetraradical XXI in 1,2-propyleneglycol which is known to freeze the biradicals described here into a stiff conformation, in hope of observing differences between the pairwise exchange interactions. This expectation did not materialize, and the 9-component spectrum of Figure 9 indicates either near-equality of exchange or that all exchange interactions are large in comparison with the hyperfine interaction. This interpretation does not agree with the observation of the molecules considered as models for the individual pairs which exhibit relatively weak exchange. This will require some understanding, since an examination of the structural formula of the tetraradical clearly shows the presence of two differently situated radical fragments (XXIa and d, and XXIb and c), whose pairwise interactions should not be equivalent. We already have some evidence for this in the fact that radicals VI, XIV, and XVII, which would model the pairwise interaction of XXIb with XXIc, show very little exchange (that is, they show a predominantly three-line spectrum). The radical XIII may be taken as a model of the pairwise interaction between XXIa and b (also XXIc and d), and it also shows only a small amount of interaction, although somewhat more than the first situation. The fact that tetraradical (XXI) seems to indicate an equivalence of the interaction of all four radical sites implies a mode of exchange transmission other than those which may go *via* pairwise interaction between XXIa and XXIc (also XXIb and XXId), and possibly XXIa and XXId as well.[37,38]

The esr spectra of the densely labeled polytyrosine (XXIII) in DMF are shown in Figure 10 (absorption spectra) and Figure 11 (first derivatives of analogous

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spectra) at two temperatures. Correlations of these spectra with other macromolecular properties will be reported elsewhere.

Conclusions

1. Procedures for the preparation of dinitroxide spin labels have been elaborated.
2. A qualitative correspondence has been established between the conformations and the esr spectra of flexible dinitroxides in various solvent systems (Table IV).
3. The intramolecular exchange interaction in the nitroxides described here is of the *direct* type.[11,21] It is effective only when the biradical molecules flex until the interacting groups are separated by a distance shorter than 6 Å.
4. The dipolar interaction introduces splitting of each line in the esr spectra of frozen samples and the dipolar splittings permit the measurement of the separation distance d between the subgroups of the biradicals. The spectra appropriate for this analysis (which should be made by comparison with computer-simulated spectra) are described in entry G, Table IV (also Figure 5). The appearance of H-type spectra (Table IV) upon freezing indicates that the biradical is coiled so that its nitroxide subunits are less than 6 Å apart.
5. None of the biradicals described here is suitable for strain gauge applications because there is no unique correspondence between the strains on the functional groups and the separation distance between the nitroxide subunits. The strain gauge would be realized by restricting the number of internal degrees of freedom.
6. Design considerations for a workable biradical strain gauge are outlined.

The Decomposition of Bis(α-naphthoyl) and α-Naphthoyl Benzoyl Peroxides

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Abstract: The thermal decomposition of bis(α-naphthoyl) peroxide in benzene and carbon tetrachloride at 60° is a mixed spontaneous and induced radical reaction. Experiments with scavengers indicate a large amount of cage recombination, the major part of which is proposed to give an α-lactone (IV) as an intermediate. IV can either react with α-naphthoyl hypoiodite (formed in the presence of iodine) to give bis(1,4-naphthoyloxy)naphthalene or rearrange to give 4-(α-naphthoyloxy)-1-naphthoic acid. α-Naphthoyl benzoyl peroxide behaves similarly, giving 4-benzoyloxy-1-naphthoic acid. The photolysis of bis(α-naphthoyl) peroxide in CCl_4 does not give 4-naphthoyloxynaphthoic acid, but does give naphthyl naphthoate, which is also one of the cage products of the thermal reaction.

The products of the decomposition of bis(α-naphthoyl) peroxide have been reported by Kharasch and Dannley[1a] and its behavior as an initiator of styrene polymerization has been described by Cooper[1b] and by Takebayashi, *et al.*[1c] The decomposition rate as measured by iodometric analysis of the peroxide in styrene is about four times that estimated from the polymerization rate,[1b,c] suggesting an unusually low efficiency in the production of free radicals. Furthermore, the rate of decomposition of bis(α-naphthoyl) peroxide appears to be almost two orders of magnitude greater than that of benzoyl peroxide[2] even after allowing for induced decomposition. The usual acceleration by *nonparticipating ortho* substituents is less than one order of magnitude, the factor of 6.5 for *o*-methyl being typical.[3a] The range of effects for *meta* and *para* substituents on the rate of decomposition of benzoyl peroxides is about a factor of 6 or 7.[3a,b]

Kinetics

The participation of radical-induced reactions in the decomposition of peroxides is usually indicated by an increased first-order rate constant at higher initial concentrations as well as by a lower rate constant in the presence of scavengers. For oversimplified models in which there is only one chain-carrying and one chain-breaking step the order n of the induced decomposition term in eq 1 varies from $^1/_2$ to 2 in steps of $^1/_2$.[3b] The observed first-order rate constant is expressed by eq 2.

$$-\frac{dp}{dt} = k_1P + k_iP^n \tag{1}$$

$$k \equiv k_1 + k_iP^{(n-1)} \tag{2}$$

For certain of these models $n = 1$ and the first-order term of eq 1 may contain a contribution from induced decomposition as well as from the spontaneous reaction. It should also be noted that because of the much greater complexity[2b] of actual chain reactions as compared with the models from which eq 1 is derived, the use of eq 1 for extrapolation to give the rate constant at infinite dilution may not be justified even though it gives an adequate fit to the rates in the range of concentrations in which the rates can actually be measured. Some

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