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Publication Date

1969-11-01

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SYNTHESIS OF MONO-, DI-, AND POLYNITROXIDES. CLASSIFICATION OF ESR SPECTRA OF FLEXIBLE DINITROXIDES DISSOLVED IN LIQUIDS AND GLASSES

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November 1969

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UNIVERSITY of CALIFORNIA BERKELEY

DISCLAIMER

Synthesis of Mono-, Di-, and Polynitroxides. Classification of ESR

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 provide guidelines for

further synthetic work. Some of the nitroxides synthesized and

characterized are: $R-CO-\overset{R_1}{N}-(CH_2)_n-\overset{R_2}{N}-CO-R$, where R is 1-oxyl-2,2,6,6-

tetramethyl pyrroline 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.

Introduction

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 nethyl-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[14,17] 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, non-covalent or ionic bonding to biological samples;
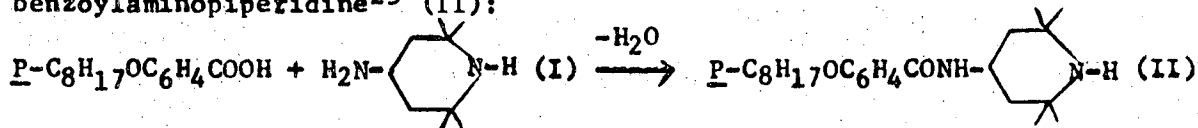
(b) modeling of the spectra expected from the biradical strain-gauge, and

(c) the synthesis of mono-, bi-, and polyradicals not directly related to the strain-gauge project.
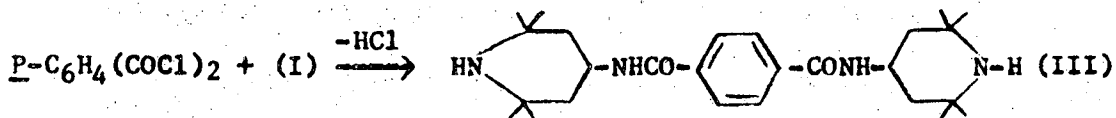
Outline of the Preparative Chemistry

Nitroxyl radicals in the piperidine series. Stable free radicals

derived from suitably substituted 1-oxyl-2,2,5,5-tetramethyl piperidine

have been useful in studying molecular ordering in nematic mesophases.[15,]

[17,18,20,25,26] 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-tetra-

methyl-4-amino piperidine (I) with p-octyloxybenzoic acid by the carbo-

diimide method[27] gave the expected 2,2,6,6-tetramethyl-4-(p-octyloxy)

benzoylaminopiperidine[25] (II):

$$\underline{P}\text{-}C_8H_{17}OC_6H_4COOH + H_2N\text{-}\langle\ \rangle\text{-}N\text{-}H \ (I) \xrightarrow{-H_2O} \underline{P}\text{-}C_8H_{17}OC_6H_4CONH\text{-}\langle\ \rangle\text{-}N\text{-}H \ (II)$$

Selective acylation of (I) with terephtaloyl chloride gave N,N-bis-4-

(2,2,6,6-tetramethyl) piperidyl terephthalamide (III):

$$\underline{P}\text{-}C_6H_4(COCl)_2 + (I) \xrightarrow{-HCl} HN\langle\ \rangle\text{-}NHCO\text{-}\langle\ \rangle\text{-}CONH\text{-}\langle\ \rangle\text{-}N\text{-}H \ (III)$$

The catalytic oxidation[28] of (II) gave radical (IV) (Table I), the

chemistry of which has been described elsewhere.[25] The catalytic oxida-

tion 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.

Nitroxyl radicals in the hydrogenated pyrrole series. We prepared

a number of mono-, bi-, and multiradicals deriving from substituted

1-oxyl-2,2,6,6-tetramethyl pyrroline.[28-31] These radicals have been

used to study nerve membranes.[24]

Table I. Piperidine-derived mono-nitroxides (IV, V) and a dinitroxide (VI).

No.	Name	Structural Formula
IV	1-oxyl-2,2,5,5-tetramethyl-4-(p-octyloxy)benzoyl amino-piperidine[22]	$\underline{n}$-C$_8$H$_{17}$O-⟨benzene⟩-CONH-⟨piperidine⟩N-O
V	N-4-(2,2,6,6-tetramethyl) piperidyl-N'-4-(1-oxyl-2,2,6,6-tetramethyl) piperidyl terephthalamide	HN⟨piperidine⟩-NHCO-⟨benzene⟩-CONH-⟨piperidine⟩N-O
VI	N,N'-bis-4-(1-oxyl-2,2,6,6-tetramethyl)piperidyl terephthalamide	O-N⟨piperidine⟩-NHCO-⟨benzene⟩-CONH-⟨piperidine⟩N-O

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.

A. Monoradicals

A radical was obtained by coupling imidazole with 1-oxyl-2,2,6,6-tetramethyl-3-carboxypyrroline (VII), the latter obtained by the procedure of Rozantsev and Krinitzkaya.[28]

(VII)   (VIII)
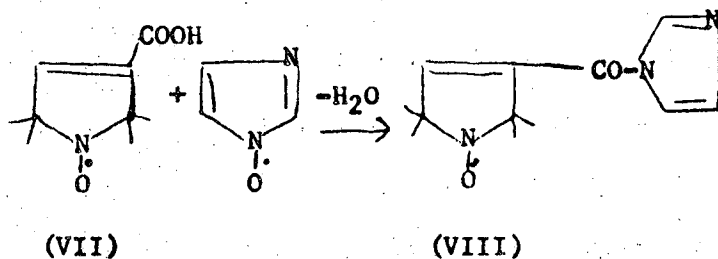
N-(1-oxyl-2,2,6,6-tetramethyl pyrroline-3-carboxy)imidazole (VIII)

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'-carbonyl diimidazole,[32] but with a lower yield.

Due to the general reactivity of the carboxylic acid.imidazolides towards 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 (IX).

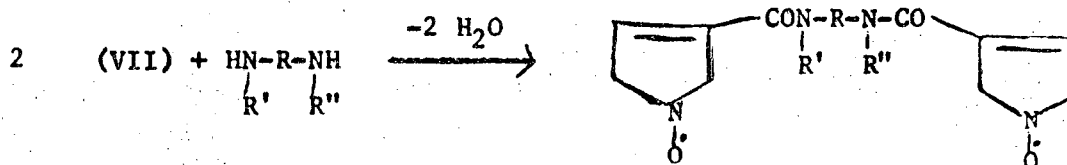$$(VIII) + H_2NCH_2COOH \xrightarrow{-C_3H_4N_2}$$
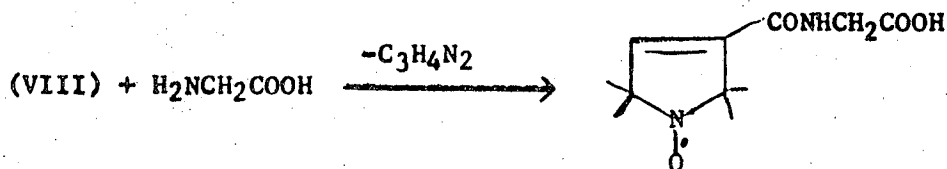
N-(1-oxyl-2,2,6,6-tetramethylpyrroline-3-carboxy) glycine (IX)

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

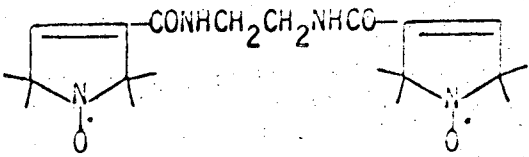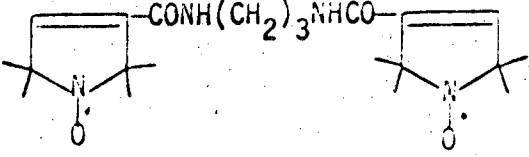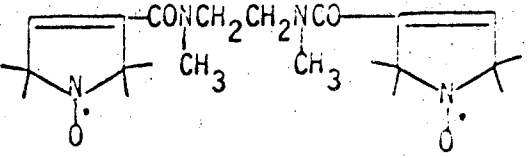of biradicals of varying physical and chemical properties:

$$2 \quad (VII) + \underset{\underset{R'}{|}}{HN}-R-\underset{\underset{R''}{|}}{NH} \quad \xrightarrow{-2\ H_2O} \quad \text{[biradical structure: } CON-R-N-CO \text{ with } R', R'' \text{ substituents]}$$
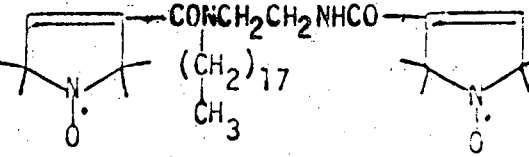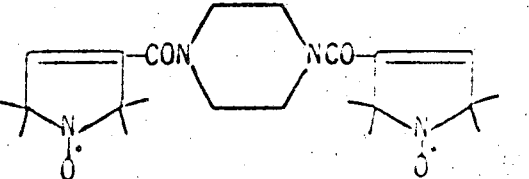
Biradicals obtained by this method, with R' and R" being H or alkyl groups and R being an alkylene chain, are listed in Table II. The ESR spectra of radicals (X), (XI), and (XIV) have been published previously.[24] The ESR spectra of radicals (XII) and (XIII) show varying degrees of steric interference 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 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) ethylene diamine-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 bis-quaternary ammonium salt (XIX). The aforementioned reactions are summarized in Table III.
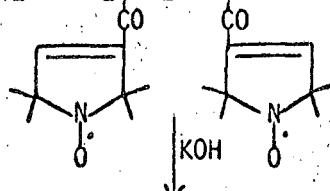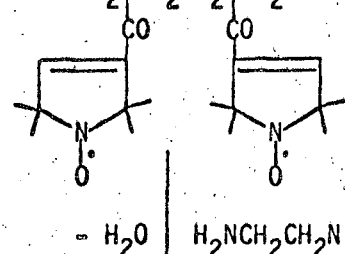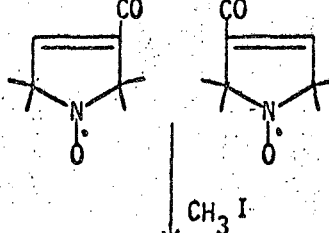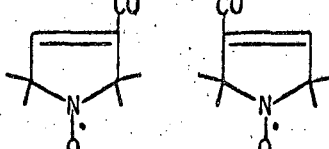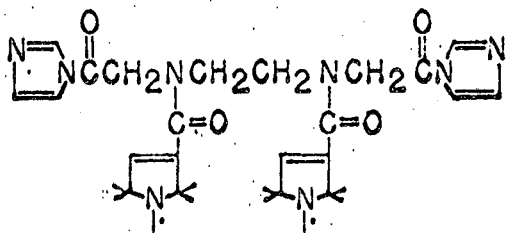
The three aforementioned radicals (XVII, XVIII, and XIX) are expected to ionize in aqueous solutions, thus providing charged functional groups. These charged groups are intended to provide coulombic interaction with charged sites of biological structures.

Table II. Lyophylic pyrroline derived dinitroxides.

No.	Name	Structural Formula
X	N,N'-bis-(1-oxyl-2,2,5,5-tetramethylpyrroline-3-carboxy)-1,2-diaminoethane	$-CONHCH_2CH_2NHCO-$
XI	N,N'-bis-(1-oxyl-2,2,5,5-tetramethylpyrroline-3-carboxy)-1,3-diaminoethane	$-CONH(CH_2)_3NHCO-$
XII	N,N'-dimethyl-N,N'-bis(1-oxyl-2,2,5,5-tetramethylpyrroline-3-carboxy)-1,2-diaminoethane	$-CONCH_2CH_2NCO-$ with CH_3 groups
XIII	N-n-octadecyl-N,N'-bis-(1-oxyl-2,2,5,5-tetramethylpyrroline-3-carboxy)-1,2-diaminoethane	$-CONCH_2CH_2NHCO-$ with $(CH_2)_{17}$ CH_3
XIV	1,4-bis-(1-oxyl-2,2,5,5-tetramethyl-3-carboxy)-piperazine	$-CON$ $NCO-$

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.

Table III.   Pyrroline-derived dinitroxides equipped with a) two ionizable groups (XVII-XIX) which would bond ionically to charged sites on macro-molecules, and b) two imidazolide groups (XX) which bond covalently to side chains of proteins.

No.	Structural Formula
XVI	$H_5C_2OCOCH_2NCH_2CH_2NCH_2COOC_2H_5$ KOH
XVII.	$HOOCCH_2NCH_2CH_2NCH_2COOH$ $- H_2O$ ↓ $H_2NCH_2CH_2N(CH_3)_2$
XVIII	$(CH_3)_2NCH_2CH_2NHCOCH_2NCH_2CH_2NCH_2CONHCH_2CH_2N(CH_3)_2$ CH_3I
XIX	$(CH_3)_3NCH_2CH_2NHCOCH_2NCH_2CH_2NCH_2CONHCH_2CH_2N(CH_3)_3$ I^- I^-
XX	

Table III (Continued)

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 ionizable groups which brings the nitroxide groups closer than in the neutral molecule, a situation described in entry D of Table IV.

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 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 triethylene-tetramine by using the carbodiimide method. The product was N,N',N'',N'''-tetra-(1-oxyl-2,2,5,5-tetramethylpyrroline-3-carboxyl) triethylene-tetramine (XXI).

$$H_2N(CH_2)_2NH(CH_2)_2NH(CH_2)_2NH_2 \quad + \quad 4 \ (VIII) \quad \xrightarrow{\ -4 \ C_3H_4N_2\ }$$

(XXI)
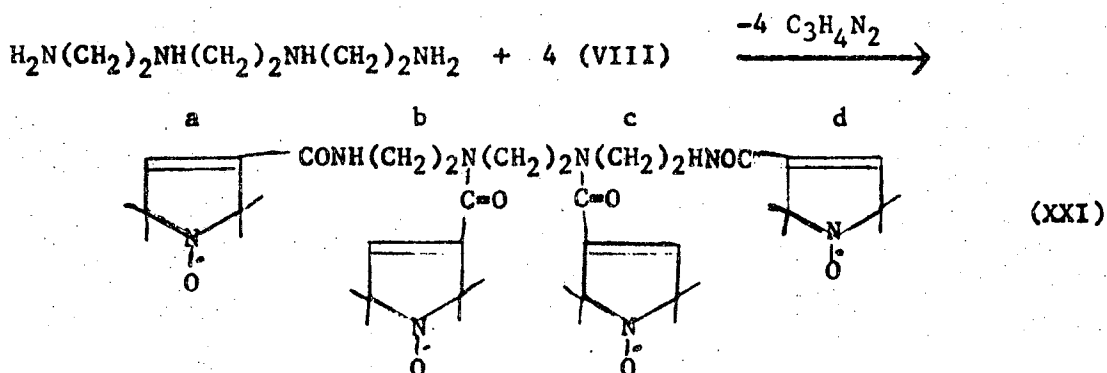
The ESR spectrum of the tetraradical is shown in Fig. 9.

Table IV. Qualitative correlation of observed ESR spectra of flexible dinitroxides with the conformations and tumbling rates in various solvents.

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

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 subdivision relates to the internal degrees of freedom which facilitate direct spin exchange by the overlap of the singly occupied orbitals in the bent biradical.

The radical imidazolide (VIII) was used as an acylating reagent to obtain <u>radical polymers</u>. In fact, treatment of suitable macromolecular compounds containing acylable 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.

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

$$(-CH_2CH_2NH-)_x + x \ (VIII) \xrightarrow{- x \ C_3H_4N_2}$$
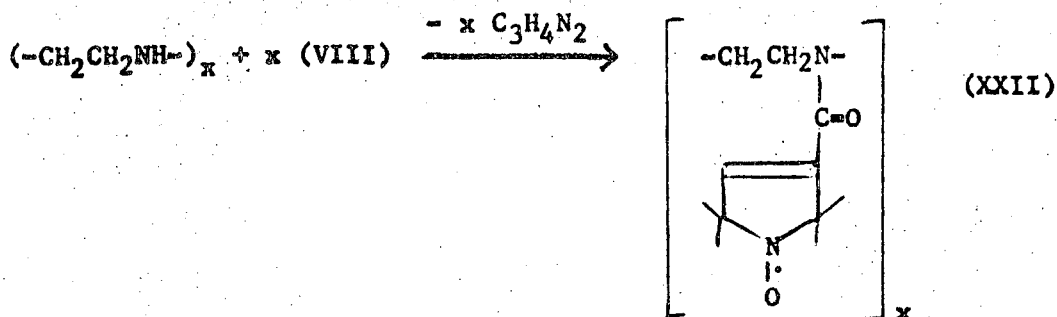
(XXII)

We have also prepared poly-O-(1-oxyl-2,2,5,5-tetramethylpyrroline-3-carboxy)-L-tyrosine (XXIII) and poly-N-(1-oxyl-2,2,5,5-tetramethylpyrroline-3-carboxyl)-L-lysine (XXIV):

$$(-NHCHCO-)_x + x \ (VIII) \xrightarrow{- x \ C_3H_4N_2}$$

(XXIII)

$$(-NHCHCO-)_x + x \ (VIII) \xrightarrow{- \ x \ C_3H_4N_2} \left[\begin{array}{c} -NHCHCO- \\ | \\ (CH_2)_7 \\ | \\ NHCO- \end{array} \right]_x \quad (XXIV)$$
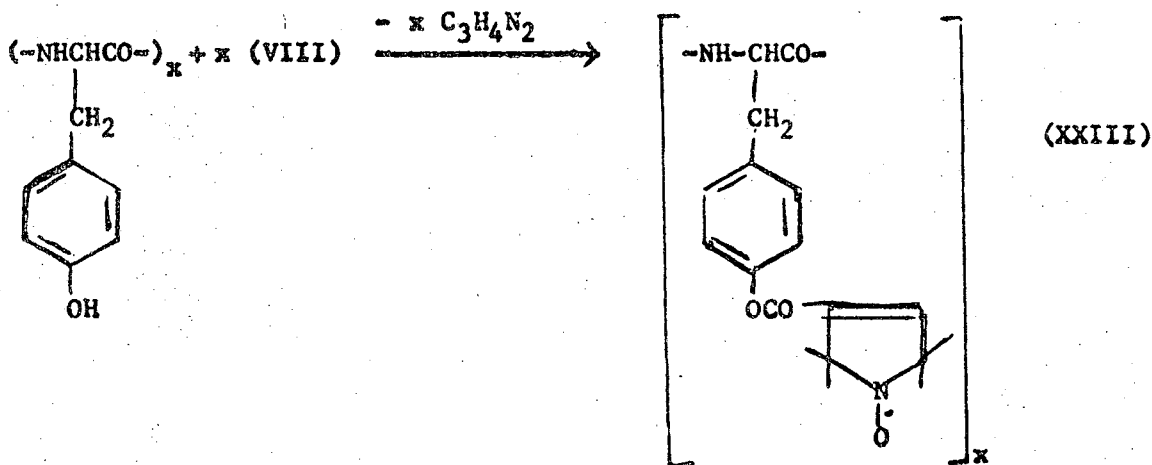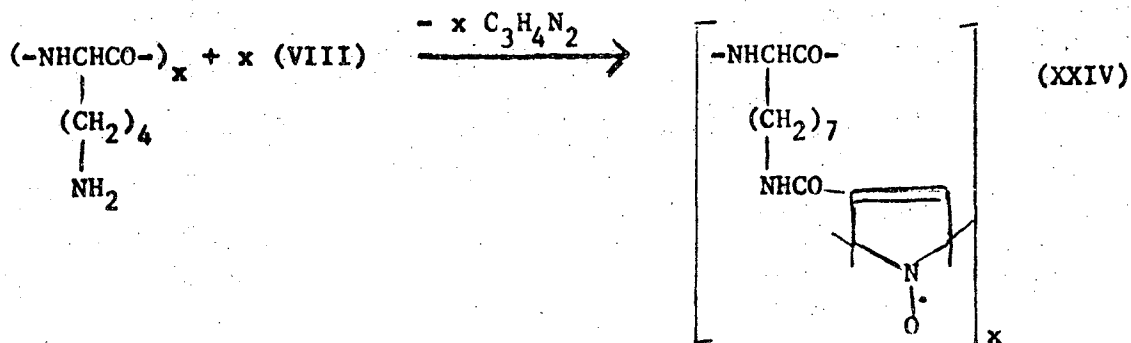
Experimental

N,N-bis-4-(2,2,6,6-tetramethyl) piperidylterephtalamide (III). A solution of 2.03 g terephtaloyl chloride in 40 ml chloroform was added dropwise into a stirred solution of 3.12 g of (I) in 35 ml chloroform, cooled at ice temperature. The reaction mixture was stirred 1 hour 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 $\underline{N}$ hydrochloric acid. The base was then precipitated with excess of sodium hydroxide, filtered and recrystallized from isopropyl alcohol.

M.P.: 308-310° (dec).

Yield: 3.0 g (68%).

Analysis: N, 12.77% ($C_{26}H_{42}N_4O_2$ contains N 12.66%).

N-4-(1-oxyl-2,2,6,6-tetramethyl) piperidyl-N'-4-(2,2,6,6-tetramethyl) piperidylterephtalamide (V) and N,N'-bis-4-(1-oxyl-2,2,6,6-tetramethyl) piperidylterephtalamide (VI). One ml of 30% hydrogen peroxide solution was added to a stirred suspension of 0.25 g of (I), 0.05 g EDTA and 0.05 g sodium tungstate in 10 ml methanol. After stirring at room temperature for 7 days, the mixture was poured into 150 ml of 0.5 $\underline{N}$ 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.

M.P.: 298-301° (dec).

Analysis: C, 67.76%; H, 9.19%; N, 11.94% ($C_{26}H_{41}N_4O_3$ contains 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.

M.P.: 274-276° (dec).

Analysis: C, 66.22%; H, 8.28%; N, 11.90% ($C_{26}H_{40}N_4O_4$ contains 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-dimethylamino-propyl) carbodiimide hydrochloride* and 0.34 g of imidazole in 30 ml chloroform was stirred at ice temperature for 1 hour, followed by 8 hours of stirring while warming at room temperature. The yellow mixture was filtered and extracted 2 times 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

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*In the descriptions which follow in subsequent preparations the term "carbodiimide" refers to this particular compound.

yellow crystalline solid, which was recrystallized from n-hexane. Yellow needles.

M.P.: 101-102°C.

Analysis: C, 61.36%; H, 7.16%; N, 17.98% ($C_{12}H_{16}N_3O_2$ contains 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 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 hour. The solution was then acidified with hydrochloric acid and extracted 3 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 crystallizes 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.

M.P.: 130-131°C.

Analysis: C, 55.09%; H, 7.21%; N, 11.26% ($C_{11}H_{17}N_2O_4$ contains 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 chloroform was stirred at ice tem-
perature for 2 hours, followed by 8 hours of stirring while warming
at room temperature. The yellow reaction mixture was filtered and
washed with water, 2 $\underline{N}$ sulfuric acid, water, 5% sodium chloride solution
before drying over anhydrous sodium sulfate. After removing the drying
agent, the filtrate was concentrated $\underline{in\ vacuo}$ to give 1.41 g (72%) of (X),
which may be recrystallized from chloroform/ethyl ether. Pale yellow
needles.

M.P.: 226-227°C (dec).

Analysis: C, 60.90%; H, 8.43%; N, 14.17% ($C_{20}H_{32}N_4O_4$ contains

C, 61.17%; H, 8.23%; N, 14.27%).

N,N'-bis(1-oxyl-2,2,5,5-tetramethylpyrroline-3-carboxy)-1,3-diamino-
propane (XI). A mixture of 0.92 g of (VII), 1.15 g carbodiimide and
0.185 g 1,3-propylenediamine in 15 ml chloroform was processed as pre-
viously described. The product was recrystallized from benzene. Yellow
powder.

M.P.: 180°C (dec).

Yield: 0.5 g (49%).

Analysis: N, 13.64% ($C_{21}H_{34}N_4O_4$ contains 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
carbodiimide and 0.11 g 1,2-bis(methylamino) ethane in 10 ml chloroform
was processed as previously described. The product was recrystallized
by dissolving in warm chloroform and diluting with excess of ether.
Pale yellow powder.

M.P.: 260°C (dec).

Yield: 0.31 g (61.5%).

Analysis: C, 62.35%; H, 8.45%; N, 12.97% ($C_{22}H_{36}N_4O_4$ contains 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 carbodiimide and 0.78 g of N-n-octadecyl-1,2-diaminoethene[33] in 30 ml chloroform and 30 ml pyridine was stirred for 1 hour at ice temperature, followed by 24 hours of stirring while warming at room temperature. The solution was then diluted further with 30 ml 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 hours. A crystalline solid, homogeneous by T.L.C. ($SiO_2/CHCl_3$) precipitated out and was recrystallized from n-hexane to give 0.35 g (21.7%) of (XIII). Pale yellow powder.

M.P.: 84-85°C.

Analysis: C, 70.45%; H, 10.71%; N, 8.77% ($C_{38}H_{68}N_4O_4$ contains 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 carbodiimide and 0.39 g of freshly recrystallized piperazine in 30 ml 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.

M.P.: 260°C (dec).

Analysis: C, 63.16%; H, 8.13%; N, 13.39% ($C_{22}H_{34}N_4O_4$ contains

C, 62.94%; H, 8.13%; N, 13.55%).

Diethyl ester of ethylenediamine-N,N'-diacetic acid (XV). A suspen-

sion of 5 g ethylenediamine-N,N'-diacetic acid (purchased from K&K) in

350 ml absolute ethyl alcohol was saturated with gaseous hydrogen

chloride and refluxed for 10 hours. An almost clear solution was

obtained. The solution was filtered while hot and then chilled in an

ice bath for 1 hour. The hydrochloride of (XV) crystallized out as

white leaflets and was recrystallized from absolute ethyl alcohol.

M.P.: 170°C (dec).

Yield: 4.4 g (50%).

Analysis: N, 9.00% ($C_{10}H_{22}N_2O_4Cl_2$ contains 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 (XVI) and 1.75 g carbodiimide in 20 ml CHCl$_3$ was stirred

1 hour at ice temperature, followed by stirring 8 hours at room tempera-

ture. 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 T.L.C.

(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 $\underline{N}$ potassium hydroxide was stirred 2 hours at 40°C; 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 discarded. The resulting clear aqueous solu-
tion 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,
1:10 (v/v) to yield 0.24 g (36.3%) of pure (XVIII). Pale yellow powder.

M.P.: 250°C (dec).

Analysis: C, 56.49%; H, 7.11%; N, 11.29% ($C_{24}H_{36}N_4O_8$ contains
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 2-dimethylaminoethylamine and 0.150 g carbodiimide-in
3 ml chloroform was stirred 1 hour at ice temperature followed by 10 hours
of stirring at room temperature. The resulting clear solution was diluted
with 15 ml chloroform, washed with water, 0.1 $\underline{N}$ 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°C. This procedure was repeated twice to yield 0.1 g (42.6%) of (XVIII) which softens at 63-65°C and melts completely at 80-82°C.

Analysis: N, 16.93% ($C_{32}H_{56}N_8O_6$ contains N 17.27%).

Dimethiodide of (XVIII), (XIX). A solution of 0.08 g of (XVIII) in 1 ml chloroform was treated with 0.1 ml methyl iodide and left at room temperature 1 hour with occasional stirring. A precipitate was formed. The suspension was diluted with 2 ml 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°C and melts completely with decomposition at 205-210°C.

Analysis: N, 11.77% ($C_{34}H_{62}N_8O_6I_2$ contains 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 triethylenetetramine, 0.491 g (VII) and 0.65 g carbodiimide in 10 ml 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.

M.P.: 216-217°C.

Analysis: N, 13.98% ($C_{42}H_{66}N_8O_8$ contains N 13.82%).

Poly-N-(1-oxyl-2,2,5,5-tetramethylpyrroline-3-carboxy) ethylene-imine (XXII). Polyethyleneimine (0.06 g) of M.W.$_n$ 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 two 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) is thoroughly washed with ether and dried at room temperature under vacuum.

Yield: 0.325 g (80.6%).

Analysis: N, 13.59% ($[C_{11}H_{17}N_2O_2]_x$ contains N 13.39%). The analysis suggests that the degree of acylation is practically quantitative (the nitrogen content of polyethyleneimine is 32.52%).

Poly-O-(1-oxyl-2,2,5,5-tetramethylpyrroline-3-carboxy)-L-tyrosine (XXIII). Poly-L-tyrosine (0.150 g) of $M.W._n$ 100,000 was dissolved in 5 ml of anhydrous dimethylformamide and 0.3 g (VIII) was added. After stirring 48 hours at room temperature the reaction mixture was poured into 50 ml ether. The precipitated (XXIII) was dissolved again in dimethylformamide and re-precipitated with excess of 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 $M.W._n$ 100,000 was dissolved in 2 ml water, and the solution was made alkaline with 0.1 ml 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 hours. The reaction mixture was then poured into a large volume of water; the precipitated

(XXIII) was thoroughly washed with water, dissolved in dimethyl formamide and re-precipitated 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.

Mono-nitroxides. The ESR spectra of isolated methyl-protected nitroxides consist of three component lines separated by the anisotropic hyperfine interaction between the electron and the 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; the range dependence of the observed interactions can be used in an inverse manner to deduce the conformation of the biradical.[17,22,24]

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

The dependence on r as well as on <u>external angular coordinates</u> 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.[17,18] 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 <u>spin exchange interaction</u> is brought about by the direct overlap of the free radical subunits, due to conformational collisions,[12-14,16] 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.

<u>Direct exchange</u> 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 r 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 $-CH_2-CH_2-$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 <u>frozen</u> 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 $-CONH-(CH_2)_2-NHCO-$ connecting chain. This chain was found to permit conformational spin exchange <u>in solution</u>. 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 <u>not</u> 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 linewidths 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 secular interactions.

The absence of exchange in the anchored biradicals indicates that
nothing short of direct collision of the NO subunits would permit this
interaction. Dipolar interaction was not observed, probably because of
the residual mobility of the biradicals.

At this point it became clear that new structural criteria have to
be defined for strain-gauge molecules.

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-r and small-r conformations and then the small-r 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):

$\underline{A}$: 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[12-21,24] (Fig. 6, lowest spectrum).

$\underline{B}$: The biradicals tumble freely but their internal degrees of freedom are frozen in a stiff conformation of maximum r, in which exchange does not occur and the dipolar interaction is minimal. The spectra consist of three lines only and are identical to those of rapidly tumbling non-interacting mono-nitroxides. 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;[15,24] b) in solutions of flexible biradicals, the bending motion of which is hindered at low temperatures;[17] c) the solutions of flexible biradicals which are stiffened by a specific interaction with the solvent[12,15] (Figs. 1,2). Gradual freezing would transform the B spectrum into an F spectrum.

$\underline{C}$: The biradicals tumble freely while being constrained in a bent (short-r) 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-r 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] 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-r conformations
in which exchange is enhanced (Fig. 4, 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 R 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 of maximum r. If the separation distance r is larger than

~18 $\overset{o}{A}$, the spectra are similar to those of non-interacting mono-nitroxides frozen in a glass. Spectra of flexible biradicals, stiffened by solvent interaction and frozen, are shown in Fig. 2, top, and Fig. 3, 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. Fig. 5, top, shows a representative spectrum, from which r 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 (Fig. 6, top). By broadly modulating the resonance of (X) in frozen paraffin wax, we have observed two unresolved, broad wings (Fig. 7). We were unable to observe the Δm=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 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 (Fig. 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 (Fig. 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 desdribed here into a stiff conforma-
tion, in hope of observing differences between the exchange interactions.
The expectation did not materialize and the 9-component spectrum indicative
of equality of exchange between the four radicals was observed as shown in
Fig. 9. 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 equiva-
lence of the interaction of all four radical sites implies a mode of
exchange transmission other than those which may go via pairwise inter-
action between (XXIa) and (XXIc) [also (XXIb and d)], and possibly
(XXIa) and (XXId) as well.[37,38]

The ESR spectra of the densely labeled polytyrosine (XXIII) in

DMF are shown in Fig. 10 (absorption spectra) and Fig. 11 (first

derivatives of analogous 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. 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. If the assumption is made that

gradual freezing of a solution does not change the conformation of

solute biradicals, the dipolar splittings permit the measurement of

the separation distance r 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 Fig. 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.

Footnotes and References

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FIGURE CAPTIONS

Fig. 1. First derivative ESR spectra of biradical X in n-octanol. The
spectra at 24°C and 40°C 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°C indicates that indirect
exchange through the backbone of the biradical molecule is
negligible. The changes in the spectra at 80°C and 120°C 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 tempera-
ture at which exchange effects appear in the spectra.

Fig. 2. First derivative ESR spectra of biradical XVII in ethylene
glycol.

a) The spectrum at -120°C represents case F in Table IV because
it is indistinguishable from that of non-interacting mono-
nitroxides frozen in a glass.

b) A B-type spectrum at room temperature.

c) Exchange was observed only after the addition of up to ∿30%
water and heating to 70°C. 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°C could produce the exchange
effects in this figure.

FIGURE CAPTIONS (Continued)

Fig. 3. 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°C fits the description of F in Table IV, the 70-100°C spectra approximate case B, and the 150°C spectrum is of A type. Heated glycerol chemically reduces the nitroxide radicals.

Fig. 4. 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.

Fig. 5. ESR spectra of XIX in glycerol. The spectrum at 20°C 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°C. The separation distance R between the nitroxide subunits can be extracted from the 20°C spectra by comparison with computer-generated spectra.

FIGURE CAPTIONS (Continued)

Fig. 6. First derivative ESR spectra of biradical X in paraffin wax. The spectrum at 24°C 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°C).

Fig. 7. ESR spectrum of biradical X in frozen paraffin wax at room temperature, taken at a large (±5 gauss) 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.

Fig. 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.

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

FIGURE CAPTIONS (Continued)

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

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

ESR SPECTRA OF BIRADICAL A IN n-OCTYL ALCOHOL

24°C

40°C

80°C

120°C

−20   3230   +20   gauss

XBL 691-4034

Fig. 1
Ferruti et al.
Synthesis of mono-, Di-, and Polynitroxide.

$-110°C$

$+24°C$

$+70°C$

30% water

−20   3230   +20   gauss
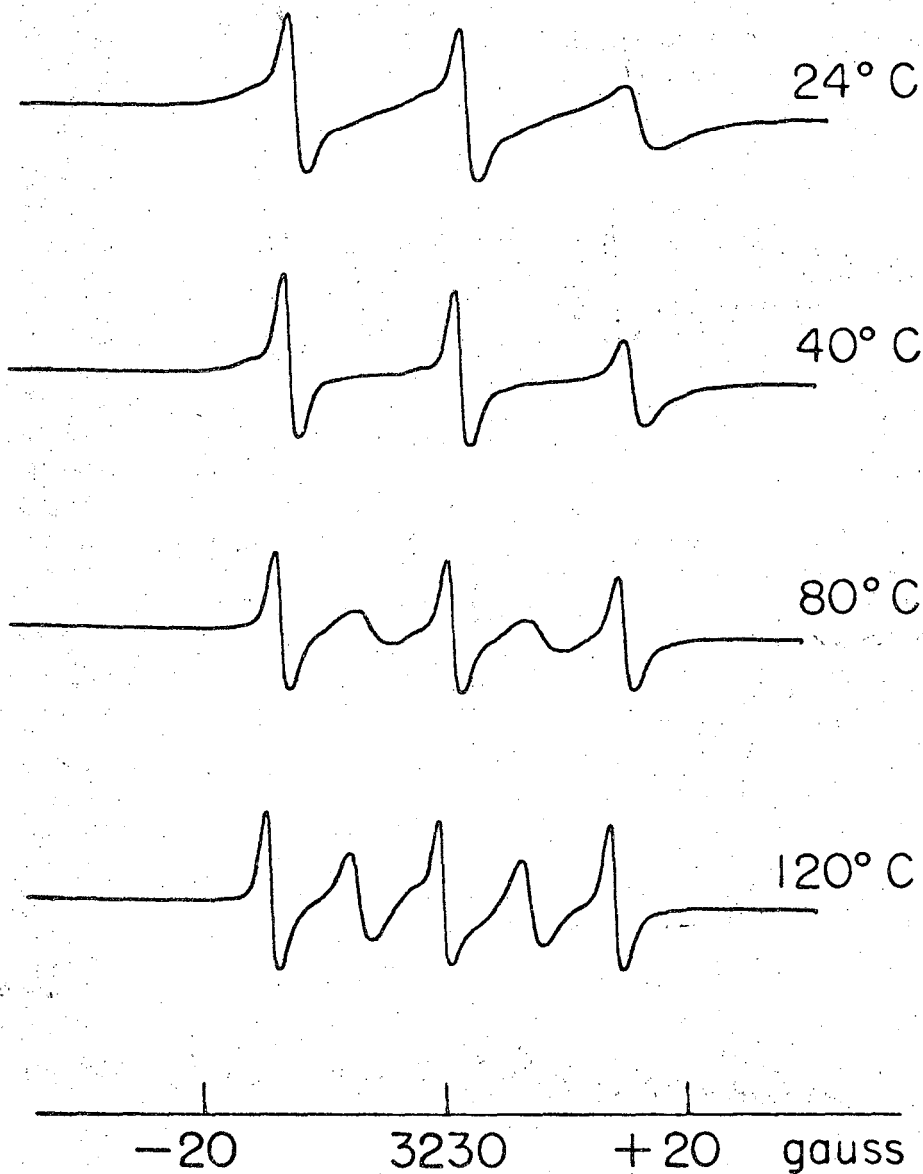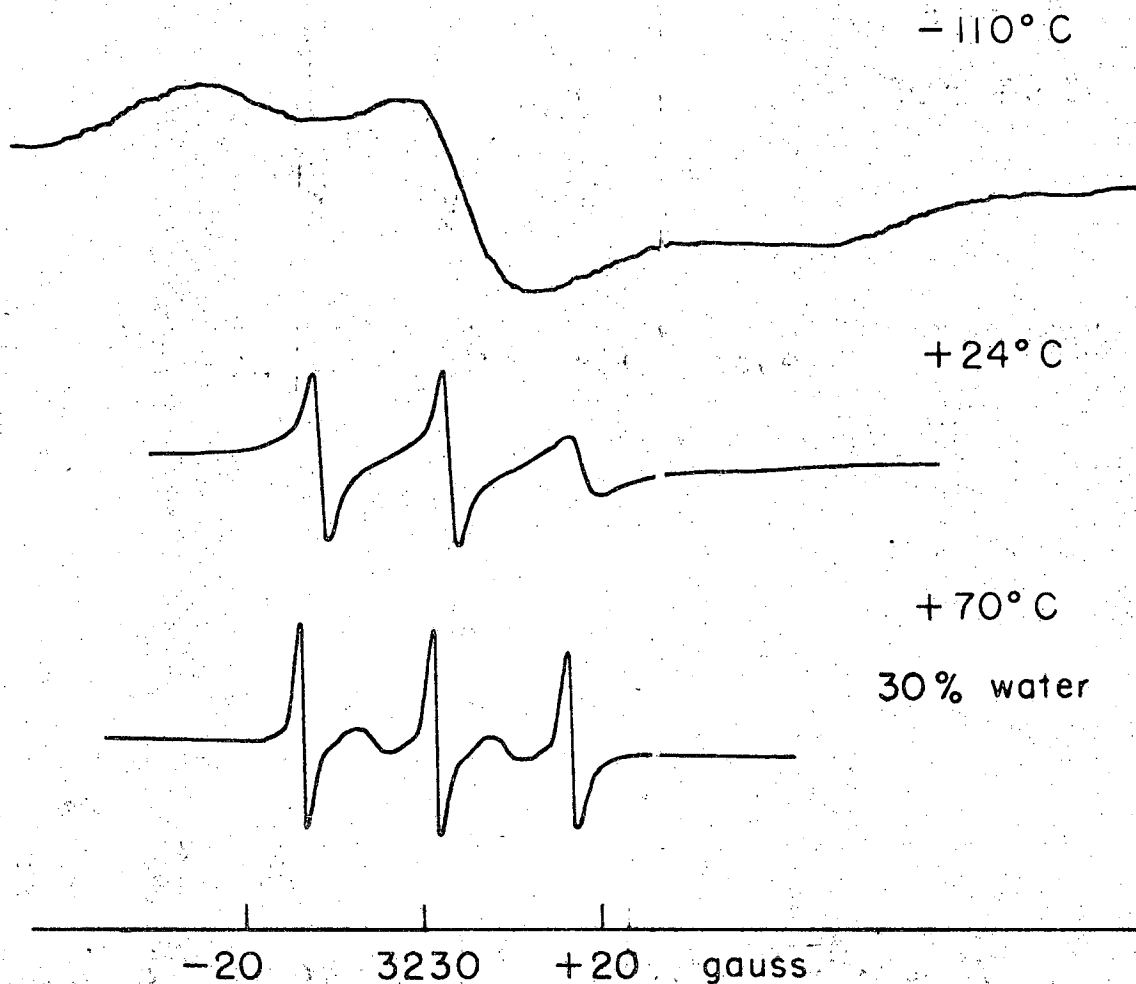
XBL 6910-5337

Fig. 2
Ferruti et al.
Synthesis of mono-, Di-, and Polynitroxides

ESR SPECTRA OF BIRADICAL A IN GLYCEROL

24°C

70°C

100°C

150°C

-20   3230   +20   +40   gauss
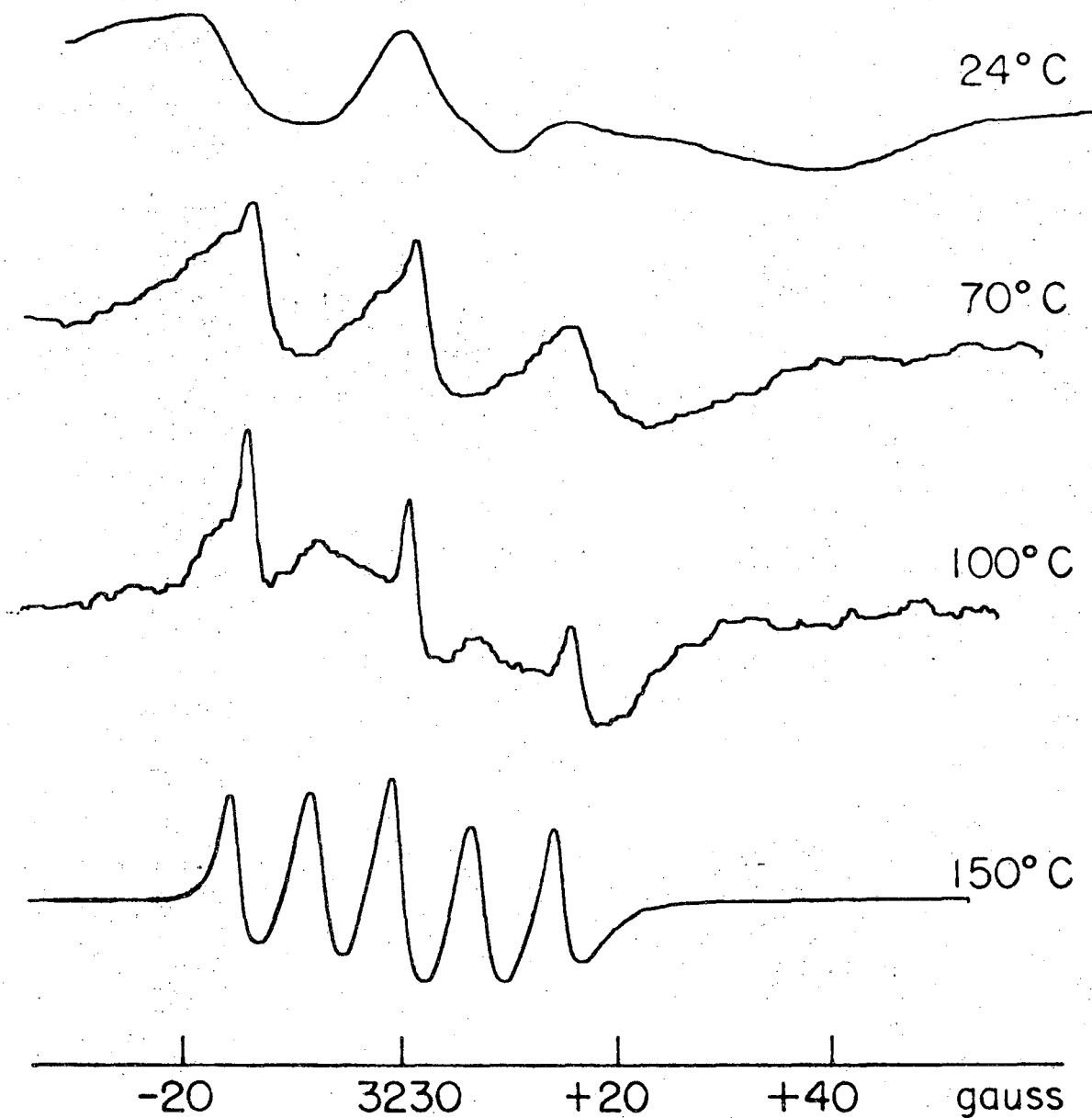
XBL 691-4039

Fig. 3
Ferruti, et al.
Synthesis of Mono-, Di-, and Polynitroxides
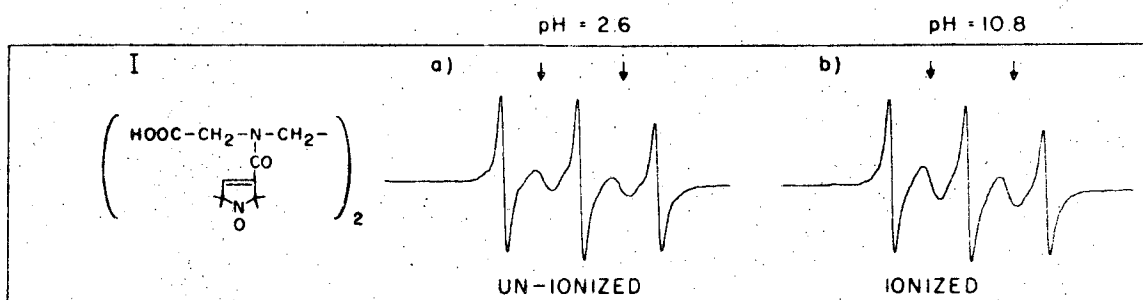
XBL 698-4371-A

Fig 4
Ferruti et al.
synthesis of mono-, Di-, and Polynitroxides
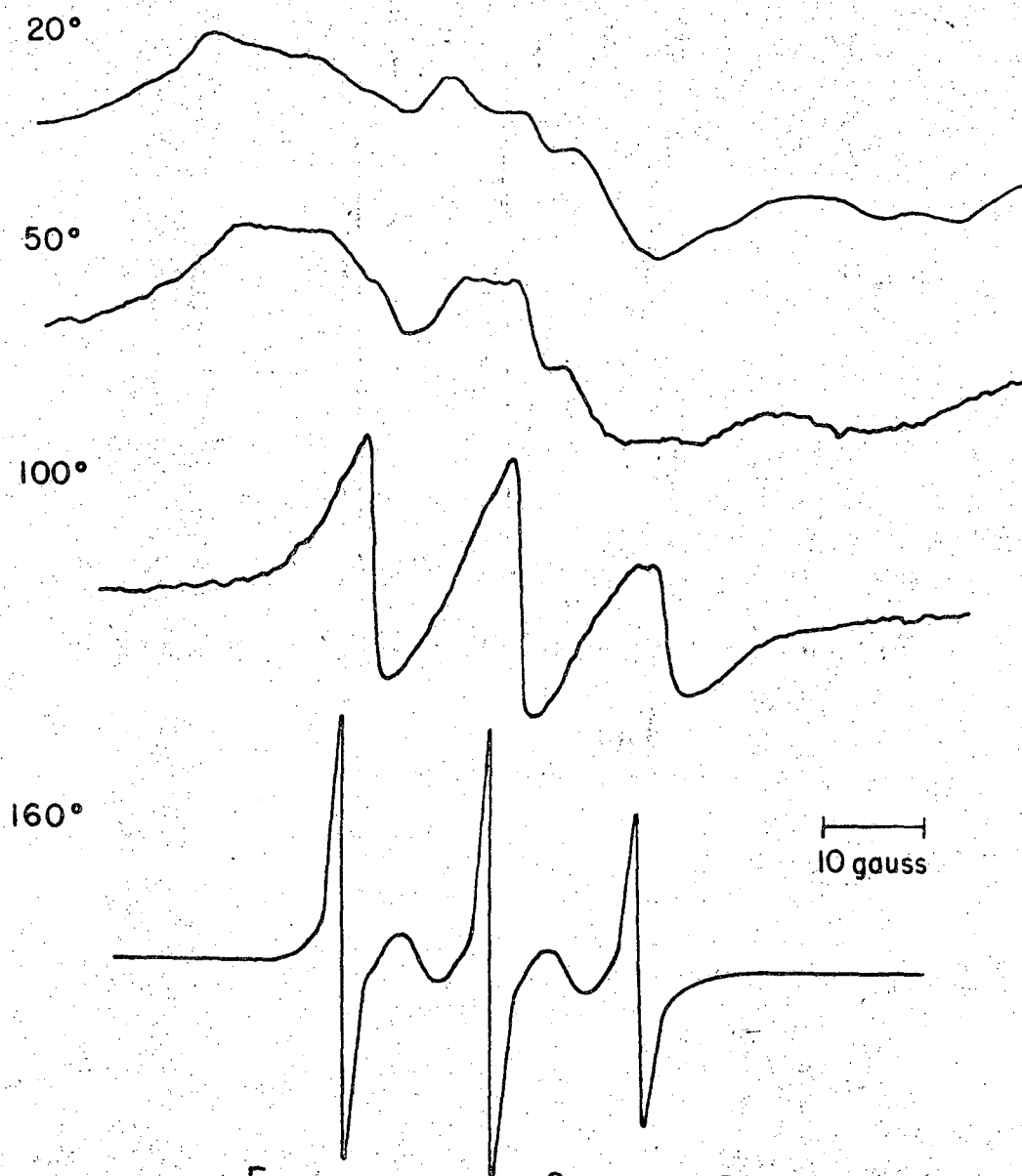
ESR SPECTRA OF $\left[(CH_3)_3\overset{I^-}{\underset{+}{N}}CH_2CH_2NH\overset{O}{\overset{\|}{C}}CH_2CH_2NCH_2- \right]_2$ IN GLYCEROL

XBL 696-4323

Fig.5

Firmiti et al.

Synthesis of Mono-, Bis-, and Polynitroxides

ESR SPECTRA OF BIRADICAL A IN PARAFFIN

24° C

80° C

180° C

-20   3230   +20   gauss
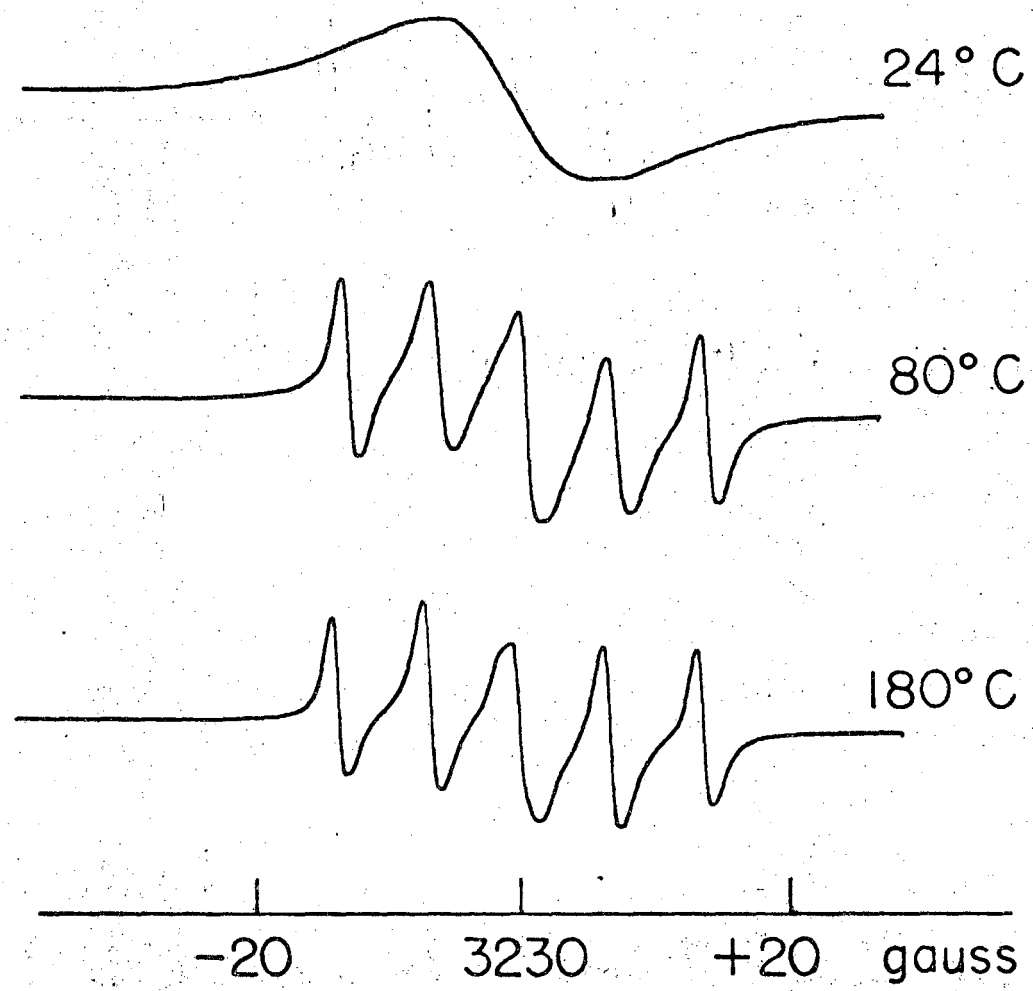
Fig. 6
Ferruti et al.
Synthesis of Mono-, Di-, and Polynitroxides

-20   3220   +20   gauss
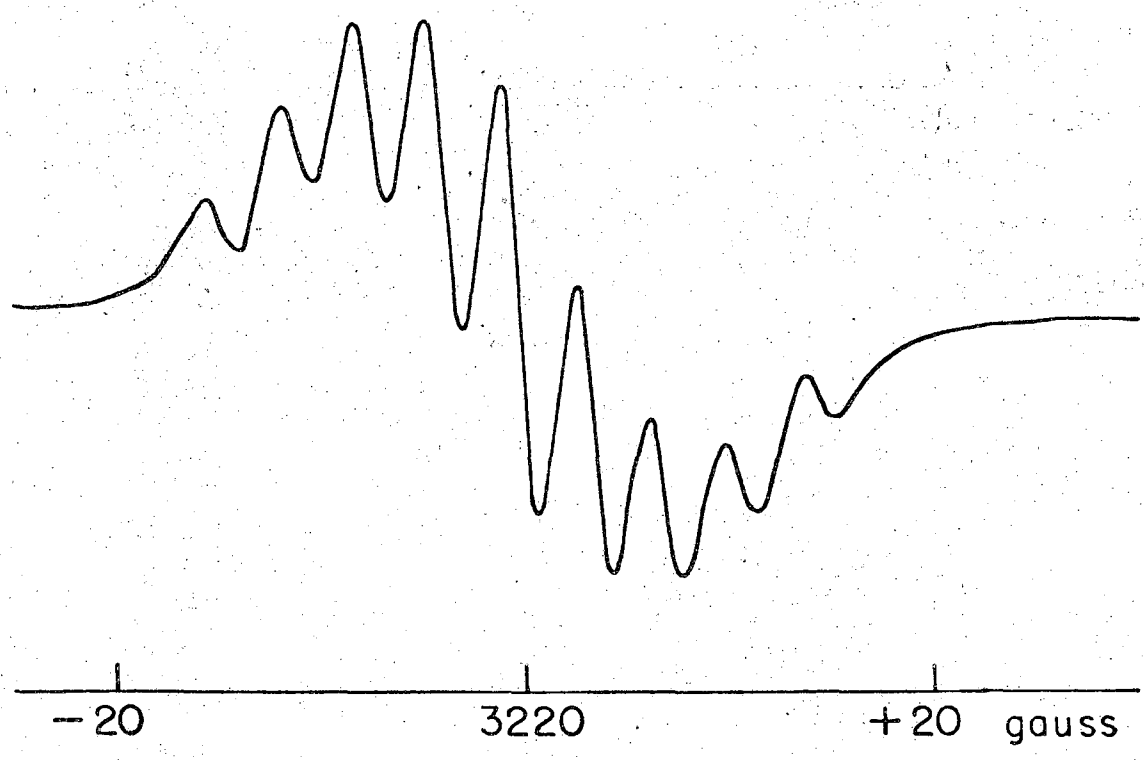
XBL 6910-5341

Fig. 9
Ferruti et al.
Synthesis of mono-, Di-, and Polynitroxides

24° C

120° C

|←——30 g——→|
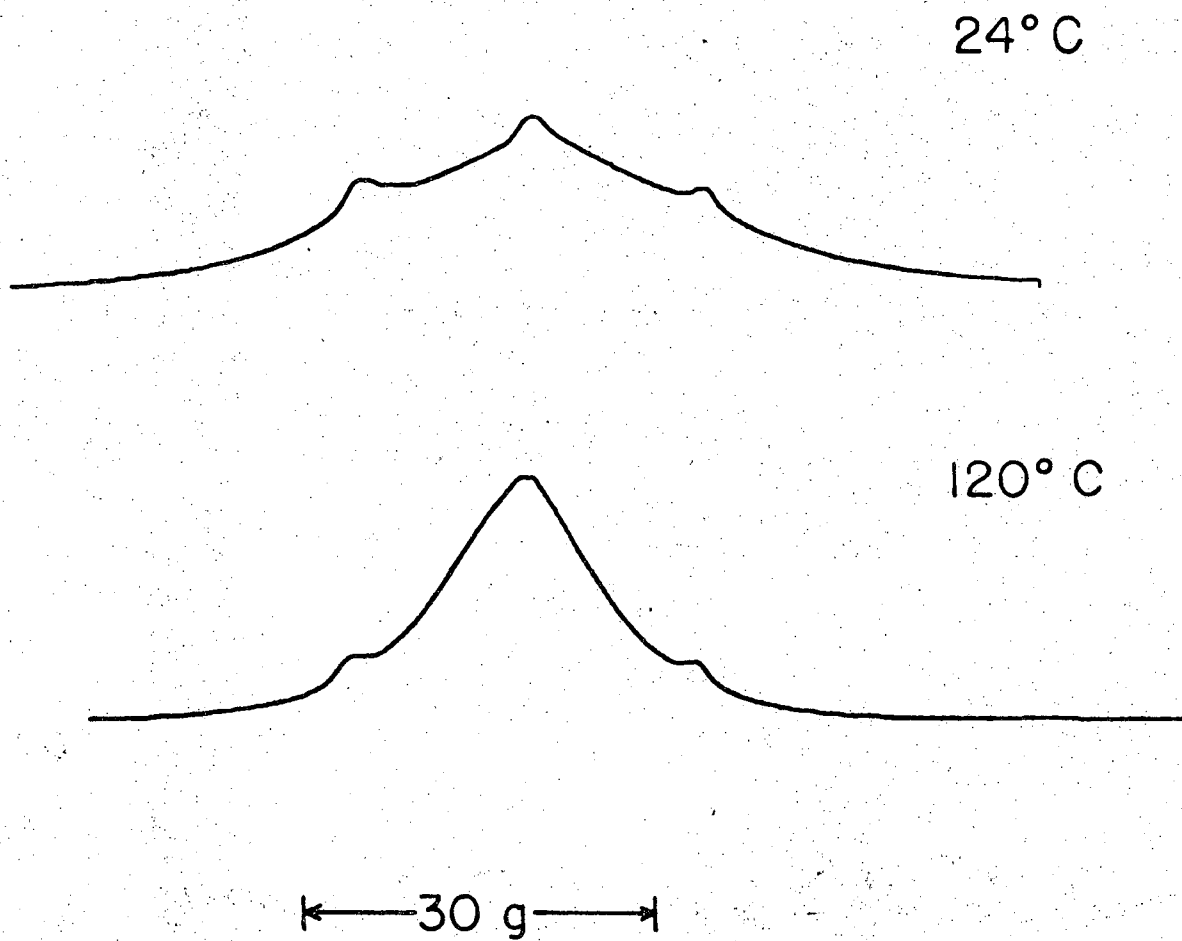
XBL 6910-5335

Fig. 10
Ferruti et al.
Synthesis of mono-, Di-, and Polynitroxides

100°C

160°C

|←——30 g——→|

XBL 6910-5334

Fig. 11
Feiruti et al.
Synthesis of mono-, Di-, and Polynitroxides