

CONFORMATIONAL STRUCTURE OF NITROXIDE BIRADICALS  
 USE OF BIRADICALS AS SPIN PROBES

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The present review deals with the problems of interpreting the experimental ESR spectra of nitroxide biradicals in dilute liquid and vitreous solutions. The problems associated with obtaining information on the structure and intramolecular motion of biradicals by the ESR method are discussed. Examples of the use of nitroxide biradicals as spin probes are examined.

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## I. INTRODUCTION

In recent years there has been a considerable increase in interest in the study of the microstructure of solutions, liquid crystals, biological membranes, and macromolecules by the spin probe (SP) method [1-7]. This method is based on the recording of the electron spin resonance (ESR) spectra of paramagnetic spin probes specially introduced into the system being studied in order to reflect various aspects of the processes taking place in the matrix. Usually, stable nitroxide monoradicals are used as probes. The theory of the ESR spectra of these radicals is well developed and makes it possible to obtain experimentally extensive information on the properties of the system being studied. The data which can be obtained by the SP method can be divided into five main groups:

- 1) the point of localization of the radical in multicomponent or heterophase systems and the polarity of its environment - from the magnitude of the hyperfine coupling constants (HFC) in liquid solutions ( $a$ ) or in solutions frozen at 77°K ( $A_{ii}$ ) [3-5];

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- 2) the rotational mobility of the radicals, the degree of anisotropy of the rotation, and the influence exerted on these factors by structural rearrangements in the system being studied – from the magnitude of the correlation time  $\tau_c$  of rotational diffusion over a wide range of frequencies [2, 4, 5] and the anisotropy parameter of the rotation  $\varepsilon$  [4, 5];
- 3) the translational mobility of the probes, the frequency of their collisions active in spin exchange  $\nu_e$ , and the influence of steric or other forms of hindrance on the magnitude of  $\nu_e$  – from the measurement of the bimolecular rate constant for spin exchange  $k_e$  [6]. The "spin labeling – spin probe" method is based on the measurement of  $k_e$  [2];
- 4) the degree of crystallinity of the frozen solution or polymer being studied [4, 7], the degree of orientation and dynamics of the structures of liquid crystals [5, 7, 8] and biomembranes [2, 5, 7–9];
- 5) the characteristic features of the spatial distribution of the probes in the matrix, the characteristic features of the crystallization of solutions and polymers – from a comparison of the local  $C_l$  and average  $C_0$  concentrations of the probes, determined from the experimentally measured values of  $k_e$  in liquid solutions [4, 11] or from the broadening of the lines of the spectrum as a result of dipole – dipole interaction between the radicals in frozen solutions [11–13].

A large number of nitroxide monoradicals which differ considerably in their solubilities in various solvents and which make it possible to carry out selective studies of various types of molecular systems have now been synthesized. An extensive group of stable nitroxide biradicals – molecules containing two paramagnetic nitroxide N–O fragments – is also well known [4, 14]. In spite of the availability of compounds of this type and the promising possibilities which they provide as spin probes, their use up to the present has been extremely restricted. The explanation is that until recently it was impossible to carry out a sufficiently complete interpretation of specific "biradical" ESR spectra.

Biradicals have a number of advantages over monoradicals as probes. By studying the motion of the molecule of the biradical as a whole or by observing its intermolecular interactions, it is possible to obtain the same data as are obtained using monoradicals. In addition, however, it also becomes possible to study the intramolecular mobility and three-dimensional structure of the biradical, and these are generally most sensitive to change in the state of the matrix. The study of intramolecular processes in biradicals makes it possible to study the following specific features:

- 6) the characteristic features of intramolecular spin exchange and the thermodynamics of intramolecular motion;
- 7) the number of conformations in which the biradical exists, and the characteristic features of transitions between the conformations when the system is subjected to different influences;
- 8) the study of dipole – dipole interaction between the paramagnetic N–O groups of the biradical makes it possible to measure sufficiently accurately the distance  $r$  between them and to observe the change in  $r$  in conformational rearrangements. In some cases it is possible, by determining  $r$  and the mutual orientation of the N–O fragments, to establish the three-dimensional structure of the molecule directly in the solution.

The present review was made necessary by the fact that published data on the study of biradicals by the ESR method are widely scattered (systematic theories of the interpretation of the ESR spectra of nitroxide biradicals have been developed only recently) and may not be familiar to research workers involved in applied studies. Existing reviews [15–17] and monographs [4–6] dealing with the problems of the interpretation of the ESR spectra of biradicals do not deal with this problem at the level of understanding now reached, with one exception [10].

The intramolecular interactions of the unpaired electrons of a biradical are usually divided into two types: anisotropic dipole – dipole interaction, which is usually the interaction of magnetic dipoles; and the independent isotropic exchange interaction, arising as a result of the overlap of the wavefunctions of the unpaired electrons [4, 10, 15, 18].

Dipole – dipole interaction can be detected only in solutions with a high viscosity, oriented structures (for example the nematic phase of liquid crystals), or in frozen solutions; sufficiently rapid rotation of the molecules of the biradicals leads to complete averaging of the dipole – dipole interaction (together with the anisotropic Zeeman and hyperfine interactions). Thus in the ESR spectra of solutions with a sufficiently low viscosity, it is possible to detect only isotropic exchange interaction.

Thus the study of the anisotropic and isotropic ESR spectra of biradicals makes it possible to obtain qualitatively different information on the molecule of the biradical. We shall subsequently examine in detail only the cases of dilute liquid solutions of low viscosity and dilute polycrystalline solutions.

In the present review we shall formulate the basic principles of the analysis of the ESR spectra of liquid and vitreous solutions of nitroxide biradicals, methods for calculating the values of the exchange integrals and the thermodynamic parameters characterizing the intramolecular motion in biradicals, and methods for measuring the distance between the N-O fragments of biradicals. We shall then examine briefly various characteristic features of the use of the molecules of biradicals as SP, and also the chief results obtained in the study of specific biological systems.

Problems associated with the study of biradicals by double electron-electron resonance [19], the electron spin echo method [20, 22], and chemically induced electron polarization [23], and also the study of biradicals by the ESR method in single crystals [17, 24-29] and liquid crystals [4, 10, 30-35] will not be discussed in the present review, because of the specific features of these methods and lack of space.

## II. STUDY OF BIRADICALS IN DILUTE LIQUID SOLUTIONS

### 1. Elements of the Theory of Electron Spin Exchange in Biradicals

It is well known [4, 5, 14] that the isotropic ESR spectra of nitroxide monoradicals in dilute liquid solutions with a low viscosity consist of three lines of approximately equal intensity and with a splitting  $a \sim 14-16$  Hz ( $\sim 3 \cdot 10^8$  rad/sec), due to the interaction of the unpaired electron of the nitrogen nucleus  $^{14}\text{N}$  (nuclear spin  $I_{\text{N}} = 1$ ). The exchange interaction of the unpaired electrons in the biradicals leads to delocalization of the unpaired electron from one radical fragment onto the other. It is natural that for a very strong exchange interaction the electrons are found to be, as it were, equally divided between the two radical centers [36]; in this case, instead of the triplet of lines characteristic of the monoradical we should observe a spectrum consisting of five components with intensity ratio 1:2:3:2:1 and with a splitting between the lines equal to  $a/2$  (Fig. 1).

This "delocalization" interpretation of a complex quantum-mechanical effect is of course extremely arbitrary. The exchange interaction can be described more correctly by the operator [18]:

$$\hat{V}_{\text{exch}} = JS^{(1)}S^{(2)}, \quad (1)$$

where  $\mathbf{S}^{(k)}$  denotes the spin operator of the  $k$ -th electron ( $\mathbf{S}^{(k)} = 1/2$ ). The quantity  $J$  is a characteristic of the "strength" of the exchange interaction and is called the exchange integral.† The value of  $J$  is determined by the overlap of the wavefunctions  $\varphi_a$  and  $\varphi_b$  of the radical fragments:

$$J = (-2) \int \int \varphi_a^*(1) \varphi_b^*(2) r_{12}^{-1} \varphi_a(2) \varphi_b(1) d\tau_1 d\tau_2$$

† Strictly speaking, in quantum mechanics the exchange integral is usually the name given to the quantity  $J_0 = -J/2$  [37].

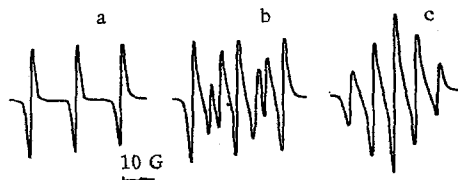


Fig. 1. Characteristic spectra of liquid toluene solutions of nitroxide mono- and biradicals: a) monoradical of 2,2',6,6'-tetramethyl-4-hydroxypiperidine-1-oxyl, b) biradical II, c) biradical XLIV.

( $r_{12}$  is the distance between electrons 1 and 2).

Generally speaking, it is not known beforehand how the overlap of the wavefunctions of the unpaired electrons takes place in the biradical (by direct overlap through a vacuum or indirectly, involving the atoms of the biradical itself or solvent molecules). Thus the determination of the mechanism of electron exchange interactions represents a separate theoretical and experimental problem.

#### a) Form of the ESR Spectrum of a Biradical with One Conformation

In the case where  $J$  is independent of time (for example the biradical exists in only one conformation), the influence of exchange interactions on the ESR spectrum of the biradical† can be calculated exactly [4, 15, 30].

Since in a liquid with low viscosity the dipole-dipole interaction of the electrons, the anisotropic hyperfine interaction, and the anisotropic Zeeman interaction are completely averaged, the spin Hamiltonian  $\mathcal{H}$  should take account of only the isotropic hyperfine and Zeeman interactions. In the most interesting situation, when the two radical fragments are identical and each have only one nucleus with nonzero nuclear spin  $I$ ,

$$\mathcal{H} = \omega_e (S_z^{(1)} + S_z^{(2)}) + a (S_z^{(1)}I_z^{(1)} + S_z^{(2)}I_z^{(2)}) + JS^{(1)}S^{(2)}, \quad (2)$$

where the spin Hamiltonian is written in frequency units, and the superscripts 1 and 2 refer to different radical fragments;  $\omega_e = g\beta_e H_0$  [4, 30].

The "correct" wavefunctions corresponding to the spin Hamiltonian (2) can be expressed in terms of the electron spin functions  $\alpha$  and  $\beta$ :

$$\begin{aligned} \psi_1 &= \alpha\alpha, \\ \psi_2 &= \frac{1}{\sqrt{2(1-\lambda^2)}} \{(\alpha\beta + \beta\alpha) + \lambda(\alpha\beta - \beta\alpha)\}, \\ \psi_3 &= \beta\beta, \\ \psi_4 &= \frac{1}{\sqrt{2(1+\lambda^2)}} \{-\lambda(\alpha\beta + \beta\alpha) + (\alpha\beta - \beta\alpha)\}, \end{aligned} \quad (3)$$

where  $\lambda = a(m_2 - m_1)/2J$  and  $m_1$  and  $m_2$  are the projections of the nuclear spins.

The energies corresponding to these wavefunctions are equal to

$$\begin{aligned} E_1 &= -g\beta_e H_0 + (1/4)J + (1/2)a(m_1 + m_2), \\ E_2 &= (1/4)J - (1/2)\{J - \sqrt{J^2 + a^2(m_1 - m_2)^2}\}, \\ E_3 &= g\beta_e H_0 + (1/4)J - (1/2)a(m_1 + m_2), \\ E_4 &= -(3/4)J + (1/2)\{J - \sqrt{J^2 + a^2(m_1 - m_2)^2}\}. \end{aligned} \quad (4)$$

For each pair of values of  $m_1$  and  $m_2$ , four transitions are observed with  $\Delta M_S = \pm 1$ : the transitions  $1 \leftrightarrow 2$  and  $2 \leftrightarrow 3$ , which correspond to transitions between "triplet" (for  $|J| \gg a |m_1 - m_2|$ ) states and which are called

† The calculation of the ESR spectra of triradicals is discussed in [31, 71, 72].

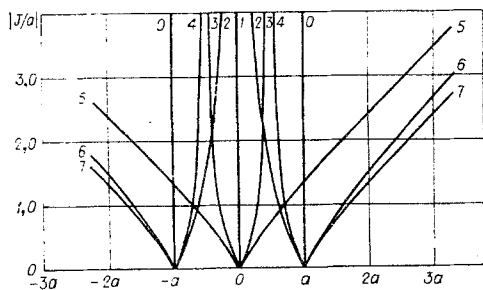


Fig. 2

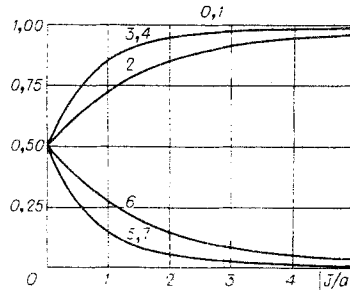


Fig. 3

Fig. 2. Dependence of the position of the lines in the ESR spectrum of a nitroxide biradical on the ratio  $|J/a|$ .

Fig. 3. Dependence of the integral intensity of the ESR lines for the nitroxide biradical on the ratio  $|J/a|$ . The figures correspond to the numbering of the components in Fig. 2. The intensity of the component "0" is taken as equal to unity.

T transitions, and transitions  $1 \leftrightarrow 4$  and  $3 \leftrightarrow 4$ , which correspond to transitions between "triplet" and "singlet" states and which are called S transitions. The frequencies of these transitions are equal to

$$\begin{aligned}\omega_T &= \omega_e - (1/2)a(m_1+m_2) \pm (1/2)(J - \sqrt{J^2 + a^2(m_1-m_2)^2}), \\ \omega_S &= \omega_e - (1/2)a(m_1+m_2) \pm (1/2)(J + \sqrt{J^2 + a^2(m_1-m_2)^2}).\end{aligned}\quad (5)$$

In the usual case for nitroxide biradicals  $|J| \ll kT$ , the intensities of the transitions are determined by the square of the matrix element  $\langle \psi_i | S_+^{(1)} + S_+^{(2)} | \psi_j \rangle$  and are equal to

$$\begin{aligned}p_T &= (1/2)\{1 + J/\sqrt{J^2 + a^2(m_1-m_2)^2}\}, \\ p_S &= (1/2)\{1 - J/\sqrt{J^2 + a^2(m_1-m_2)^2}\}\end{aligned}\quad (6)$$

(the total intensity of all four transitions is constant and equal to two) [4].

It may be noted that the position of the components of the isotropic ESR spectra of biradicals depends only on the absolute magnitude of the ratio  $|J/a|$ ; experimentally, the sign of this ratio can be found only by analyzing the anisotropic ESR spectra [30].†

From Eqs. (5) and (6) it follows that the isotropic ESR spectra of biradicals with identical radical fragments should always contain components corresponding to the ESR spectrum of the noninteracting radical fragment. These components correspond to the situation  $m_1 = m_2$ , and their intensity is independent of the magnitude of  $J$  and amounts to  $1/9$  of the total integral intensity of the spectrum. We shall call these components "basic" or "monoradical." The characteristic components, which have positions different from those of the main components and which are due to the existence of exchange interaction, are usually called "biradical" [4].

The analytical expressions making it possible to determine the magnitude of  $|J|$  from the position of the biradical components in the experimental spectrum are simple and can readily be found independently from Eqs. (5). For convenience we give in Fig. 2 a graph showing the dependence of the position of the lines in the ESR spectrum of a nitroxide biradical on the magnitude of  $|J/a|$ . The intensity of the separate lines of the spectrum is best compared with the intensity of the basic lines "0" (see Fig. 2). Figure 3 gives a graphical representation of the dependence of these relative intensities of the lines in the spectrum on the magnitude of  $|J/a|$ ; this relationship was obtained by means of Eqs. (6).

It should be emphasized that for the correct determination of  $|J/a|$ , one value of  $|J|$  should correctly describe the position and integral intensities for all lines of the spectrum simultaneously.

#### b. Model of the Intramolecular Motion of a Biradical [45]

The case of constant  $J$  examined above cannot often be applied directly to the description of real biradicals. In fact, since the exchange interaction is short-lived and its magnitude is determined by the overlap of the wavefunctions of the unpaired electrons, even small displacements of the atoms in the molecule during thermal motion should lead to appreciable modulation of the value of  $J$ , and this in turn should influence the form of the ESR spectrum.

A useful and apparently sufficiently accurate model of intramolecular motion in a biradical is that in which it is assumed that the biradical has a definite number  $N$  of conformations, and that each conformation "j" can be characterized by an average lifetime  $\tau_j$  and a relative concentration  $P_j$  (that is, the proportions of the molecules present at a given moment of time in the conformation "j"), and that the transitions from the conformation "j" to another conformation "l" takes place abruptly with an arbitrary probability  $p_{jl}$ .

For the quantities  $P_j$  and  $p_{jl}$  defined in this way, the following normalization conditions should be fulfilled:

$$\sum_{j=1}^N P_j = 1, \quad (7)$$

† In the limiting situation, when  $|J| \gg a$  and in addition the absolute value of  $J$  is comparable with the thermal energy ( $|J| \sim kT$ ), the sign and magnitude of  $J$  can be determined by studying the temperature dependence of the paramagnetic susceptibility of the specimen or the integral intensity of the ESR spectrum of the biradical [24-27, 38-43]. In fact, under these conditions a signal is observed only from the triplet state of the biradical; the probability  $p_T$  of being found in the triplet state is readily obtained from the condition for thermal equilibrium:

$$p_T = 3/(3 + \exp(J/kT)).$$

The integral intensity ( $I$ ) of the ESR signal will then be  $I \sim p_T/T$  (see [44]).

$$\sum_{l=1, l \neq j}^N p_{jl} = 1. \quad (8)$$

The quantities  $\tau$ ,  $P$ , and  $p$  are not independent parameters, since in the stationary case for conformational transitions the principle of detailed equilibrium should be fulfilled:

$$P_j p_{jl} / \tau_j = P_l p_{lj} / \tau_l. \quad (9)$$

Thus Eqs. (7)-(9) show that for a complete description of the intramolecular conformational motion of the biradical with  $N$  conformations it is necessary to know  $(2N - 1)$  independent dynamic parameters. Subsequently, we shall use the values of  $P_j$  and  $\tau_j$  as these parameters.

It should be noted that in fact the number of independent dynamic variables may be slightly smaller. Thus for  $N=2$ ,  $P_1/P_2 = \tau_1/\tau_2$ , so that, for example, it is possible to take as independent quantities only  $\tau_1$  and  $\tau_2$ .

In another case important for subsequent analysis, where  $N=3$ , we readily obtain from Eqs. (7)-(9)

$$p_{jl} = (P_j/\tau_j + P_l/\tau_l - P_n/\tau_n) \tau_j / 2P_j. \quad (10)$$

(Here and subsequently,  $j, l, n=1, 2, 3$ ;  $j \neq l, n$ ;  $l \neq n$ ).

Since the condition  $p_{jl} \geq 0$  must be fulfilled, we have for the values of  $P_j/\tau_j$  the "triangle inequality"

$$P_l/\tau_l + P_n/\tau_n \geq P_j/\tau_j \geq |P_l/\tau_l - P_n/\tau_n|, \quad (11)$$

which in a number of cases (for example for  $P_n/\tau_n \ll P_l/\tau_l$ ) can effectively decrease the number of independent dynamic parameters.

A decrease in the effective number of dynamic parameters can undoubtedly also be expected for the case where  $N > 3$ .

### c. Calculation of the Form of the ESR Spectrum of Dynamic Biradical Systems

Within the framework of this dynamic model of the biradical we can readily carry out the complete calculation of the form of the ESR spectrum. We assume that each conformation corresponds to a definite value of the exchange integral  $J_j$ , and that  $|J_j| \ll kT$ , that is, that exchange interactions do not influence the dynamics of the conformational transitions. The dynamic equations for the combined (paired) spin density matrix  $\rho_j$ , describing the  $j$ -th conformation, can then be written in the following form [18, 45, 46]:

$$\dot{\rho}_j = \sum_{l=1}^N \frac{\rho_l - \rho_j}{\tau_j} p_{jl} - \hat{R} \rho_j + i[\rho_j, \mathcal{H}_j]. \quad (12)$$

In a rotating system of coordinates, the spin Hamiltonian of the conformations is

$$\mathcal{H}_j = (\omega_e^{(1)} - \omega) S_z^{(1)} + (\omega_e^{(2)} - \omega) S_z^{(2)} + a_j^{(1)} I_z^{(1)} S_z^{(1)} + a_j^{(2)} I_z^{(2)} S_z^{(2)} + J_j S^{(1)} S^{(2)} + \omega_S^{(1)} S_x^{(1)} + \omega_S^{(2)} S_x^{(2)}, \quad (13)$$

where  $\omega_e^{(k)}$  is the resonance frequency of the electron;  $a_j^{(k)}$  is the HFI constant of the spectrum in the conformation  $j$ ; and  $\omega_S^{(k)}$  is the Zeeman frequency of the electron in the radiofrequency field ( $k=1, 2$  gives the numbering of the radical centers). The operator  $\hat{R}$  takes account of the linewidth  $(1/T_2)_0^{(j)}$  not related to spin exchange.

Without going into the details of the calculation [45, 46], we would point out that the form of the ESR spectrum is given by the expression

$$A = Im \sum_{j=1}^N P_j \sum_{m_1=-I^{(1)}}^{I^{(1)}} \sum_{m_2=-I^{(2)}}^{I^{(2)}} \left( \overset{+}{b}_{m_1 m_2}^{(j)} + \bar{b}_{m_1 m_2}^{(j)} + \overset{+}{c}_{m_1 m_2}^{(j)} + \bar{c}_{m_1 m_2}^{(j)} \right), \quad (14)$$

where the quantities  $\overset{+}{b}_{m_1 m_2}$ ,  $\bar{b}_{m_1 m_2}$ ,  $\overset{+}{c}_{m_1 m_2}$ , and  $\bar{c}_{m_1 m_2}$  can be found by solving the linear system of equations

$$\left\{ \begin{array}{l} \pm \frac{b_{m_1 m_2}^{(j)}}{\delta_{m_1 m_2}^{(j)}} (1 + \eta_j + i(\Delta_{m_1}^{(j)} \pm u_j)) - \sum_{\substack{l=1 \\ l \neq j}}^N \pm \frac{b_{m_1 m_1}^{(l)} p_{jl}}{b_{m_1 m_1}^{(l)}} \mp i u_j \frac{c_{m_1 m_2}^{(j)}}{b_{m_1 m_2}^{(j)}} = i X \tau_j, \\ \pm \frac{c_{m_1 m_2}^{(j)}}{c_{m_1 m_2}^{(j)}} (1 + \eta_j + i(\Delta_{m_2}^{(j)} \pm u_j)) - \sum_{\substack{l=1 \\ l \neq j}}^N \pm \frac{c_{m_1 m_2}^{(l)} p_{jl}}{c_{m_1 m_2}^{(l)}} \mp i u_j \frac{b_{m_1 m_2}^{(j)}}{c_{m_1 m_2}^{(j)}} = i X \tau_j. \end{array} \right. \quad (15)^\dagger$$

Here,

$$\begin{aligned} \Delta_{m_k}^{(j)} &\equiv \tau_j (\omega_e^{(k)} + a^{(k)} m_k - \omega)^*, & \eta_j &\equiv \tau_j (T_2)_0^{(j)}, \\ u_j &\equiv J_j \tau_j / 2, & X &\equiv \omega_e \text{th}(\omega_e / 2kT) / (2I^{(1)} + 1)(2I^{(2)} + 1). \end{aligned}$$

It can readily be seen that when there is intramolecular motion, the overall ESR spectrum of the biradical, as in the static case, consists of the superposition of the solutions of the system of equations (15) for all possible combinations of the pairs  $m_1$  and  $m_2$ . Thus for an analysis of the influence of conformational transitions on the form of the spectrum it is sufficient to examine only one pair of values  $m_1$  and  $m_2$ ; for a fixed value  $m_1$  and  $m_2$  the biradical can be regarded as a system containing unpaired electrons with fixed resonance frequencies:

$$\omega_1 = \omega_e^{(1)} + a^{(1)} m_1, \quad \omega_2 = \omega_e^{(2)} + a^{(2)} m_2; \quad (16)$$

in the absence of exchange interactions the corresponding ESR spectrum will consist of two lines with positions  $\omega_1$  and  $\omega_2$ .

The influence of intramolecular motion on the form of the ESR spectrum can be demonstrated most clearly for the case of the simplest situation when the hypothetical biradical has only two conformations "1" and "2" and its dynamics are described only by the parameters  $\tau_1$  and  $\tau_2$  [46].

Analysis of the solution of Eqs. (15) makes it possible to distinguish two ranges of values of the parameters  $\tau_1$  and  $\tau_2$  in which the structure of the ESR spectra of the biradical shows characteristic qualitative features.

† Equations (15) make it possible to take account of the possibility of change in the value of the constant  $a$  of the isotropic hyperfine interaction with change in the value of the exchange integral  $J$ . Nevertheless, we shall not discuss a number of interesting phenomena which would be observed if there existed a correlation between  $a$  and  $J$ , since at present there are no experimental data on the existence of a correlation of this kind.

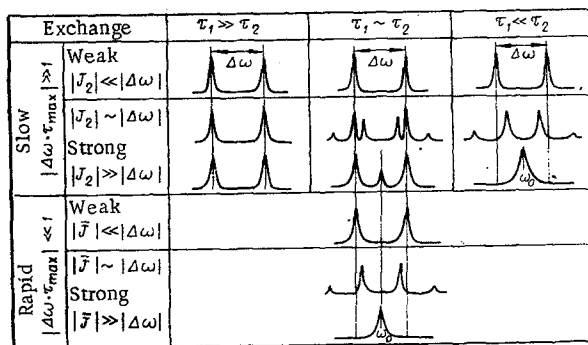


Fig. 4. Dependence of the form of the ESR spectrum of a hypothetical biradical made up of two radicals with resonance frequencies  $\omega_e^{(1)}$  and  $\omega_e^{(2)}$  without HFI on the strength and rate of exchange. For slow exchange it is assumed that  $|J_1| \ll \Delta\omega$ .

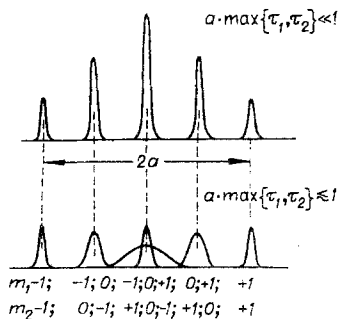


Fig. 5

Fig. 5. Alternated broadening of the lines of a biradical with two conformations and  $|\bar{J}| \gg a$ .

Conformation	Life-time	Chemical exchange	Spin exchange ( $\tau_2 \ll \tau_1, J_2^2 \tau_2^2 \gg 1, J_1 = 0,  J_2  \gg \Delta\omega$ )
1	$\tau_1$		
2	$\tau_2$		

Fig. 6

Fig. 6. Comparison of the effects of chemical and spin exchange. The heavy arrows show what must be regarded as exchange processes (it is assumed that the conditions for strongly dephasing exchange are fulfilled  $(J_2 \tau_2)^2 \geq 1$ ).  $P_{ij}$  is the probability of a transition between the corresponding energy levels, and  $F$  is a function of  $J_2$  and  $\tau_2$ .

Thus in the case where

$$\Delta\omega \cdot \max\{\tau_1, \tau_2\} > 1 \quad (17)$$

( $\Delta\omega \equiv |\omega_1 - \omega_2|$ ), the frequency of the intramolecular motion is too small to have any significant influence on the ESR spectra of the individual conformations. In this case the position and intensities of the components of the spectra of the separate conformations are described by the static relationships (5) and (6), and the overall ESR spectrum of the biradical consists of the superposition of the spectra of the two conformations; the superposition takes account of the corresponding statistical weights  $P_1$  and  $P_2$  of the conformations (in this case the statistical weight is also proportional to the values of  $\tau_1$  and  $\tau_2$ ). The widths of all the components of the spectrum for slow motion are practically independent of  $\tau$  and equal to one another.

In the case where

$$\Delta\omega \cdot \max\{\tau_1, \tau_2\} < 1, \quad (18)$$

rapid intramolecular motion leads to averaging of the ESR spectra of the two conformations, and we observe the ESR spectrum of one "effective" conformation, and the ESR parameters of this spectrum are average values, taking account of the statistical weights of the different conformations. Thus the position of the lines in the spectrum is described by the average exchange integral

$$\bar{J} = \frac{J_1 P_1 - J_2 P_2}{P_1 + P_2} = \frac{J_1 \tau_1 + J_2 \tau_2}{\tau_1 + \tau_2}. \quad (19)$$

Thus the interpretation of the ESR spectra of biradicals should depend to a significant extent on whether condition (17) or condition (18) is satisfied. In accordance with the physical significance, these conditions are conveniently described as the conditions of slow and rapid exchange respectively.

For the qualitative characterization of the form of the ESR spectrum of the biradical, it is also convenient to use the concept of strength of exchange. Thus for strong exchange  $|J_j| \gg \Delta\omega$  (for slow exchange) or  $|\bar{J}_j| \gg \Delta\omega$  (for rapid exchange), the ESR spectrum being discussed generally shows more than two lines. For weak exchange ( $|J_j| \ll \Delta\omega$  or  $|\bar{J}| \ll \Delta\omega$ ), only two "monoradical" lines are observed.

A complete scheme for this classification of the ESR spectra of the hypothetical biradical being discussed according to the rate and strength of the exchange interaction is given in Fig. 4 [46].

Rapid exchange is associated not only with averaging of the exchange integral but also with another important phenomenon, familiar in the literature as alternated broadening of the HFS components of the biradical [45, 47, 48]. We shall consider this effect in slightly more detail.

In real biradicals, as a result of the existence of HFI, the value of the quantity  $\Delta\omega$  will be different for different combinations of the projections of the nuclear spins  $m_1$  and  $m_2$ ; the extent to which conditions (17) and (18) are fulfilled will therefore also be different. It is easy to see how this influences the form of the ESR spectrum.



For the case where

$$\Delta\omega \cdot \max\{\tau_1, \tau_2\} \leq 1, \quad (18a)$$

in spite of the fact that the condition for rapid exchange is fulfilled, the averaging of the spectra of the two conformations is not complete. Thus the lines will be extensively broadened, in spite of the fact that their position is described by the averaged value  $\bar{J}$ .

For the case where

$$\Delta\omega \cdot \max\{\tau_1, \tau_2\} \ll 1 \quad (18b)$$

(which is always fulfilled for the "basic" components for which  $\Delta\omega = 0$ ) complete averaging is observed, and the width of the ESR lines is independent of the relationship between  $\tau$  and  $\Delta\omega$  and is determined by a factor which is not related to conformational transitions.

Thus in the case of rapid exchange the biradical lines due to those combinations  $m_1$  and  $m_2$  for which  $\Delta\omega$  is greatest are also broadened to the greatest extent. With increase in the rate of exchange, all the biradical lines become narrower, and their alternated broadening disappears (Fig. 5).

Numerical calculations show that appreciable alternated broadening does not alter the integral intensity of the components of the spectrum relative to the values calculated from the static equations (6) (but naturally using the value of  $\bar{J}$ ) [47, 48].

The problem of the analytical calculation of the width of the ESR lines showing alternated broadening for a two-conformation biradical has been discussed many times in the literature [10, 47-50], but it cannot yet be regarded as fully solved. This is due to the fact that all the works cited discussed the effect of alternated broadening within the framework of Redfield's relaxation equations [4, 51], which are applicable only in the case where the time theory of perturbation is applicable. For biradicals with electron spin exchange this is equivalent to the condition that the change in the phase of the precession of the spins as a result of the existence of the biradicals in the conformation "j" with  $J_j$  (equal in order of magnitude to the magnitude of the product  $J_j\tau_j$ ) is small; in other words, the condition of weakly dephasing exchange

$$J_j^2\tau_j^2 \ll 1$$

should be fulfilled. In this case the broadening of the lines can be calculated using the spectral density function

$$j(\Omega) = \frac{1}{2} \int_{-\infty}^{+\infty} [J(t) - \bar{J}][J(t) - \bar{J}] \cos \Omega t dt,$$

where  $J(t)$  is the value of the magnitude of the exchange integral at the moment of time  $t$ , which depends on the model of intramolecular motion used [4, 10, 47-50].

The broadening of the T lines is described by the expression [50]

$$\Delta(1/T_2) = j(0) \sin^4 \Phi + j(\Delta\Omega) \sin^2 2\Phi/4, \quad (20)$$

and that of the S lines by the expression

$$\Delta(1/T_2) = j(0) \cos^4 \Phi + j(\Delta\Omega) \sin^2 2\Phi/4, \quad (21)$$

where  $\tan 2\Phi = a(m_1 - m_2)/\bar{J}$ ,

$$\Delta\Omega = \bar{J} \cos 2\Phi + a(m_1 - m_2) \sin 2\Phi.$$

For many biradicals having typical "biradical" spectra with  $|\bar{J}| \gg a$ ,  $|\Phi| < 45^\circ$  so that the coefficient of  $j(0)$  in Eq. (20) is smaller than that in Eq. (21). Thus the width of the S lines is more sensitive to modulation than the width of the T lines.

In the case where rapid exchange takes place between two conformations A and B [4],

$$j(\Delta\Omega) = (J_A - J_B)^2 \frac{\tau_A\tau_B}{(\tau_A + \tau_B)^2} \frac{\tau}{1 + \Omega^2\tau^2}, \quad (22)$$

where

$$\tau = \tau_A\tau_B/(\tau_A + \tau_B).$$

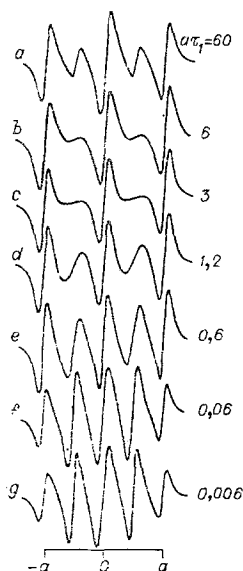


Fig. 7

Fig. 7. Influence of the rate of exchange on the form of the ESR spectrum of a biradical with two conformations.  $\tau_2/\tau_1=1$ ,  $J_1=0$ ,  $J_2=33a$ ,  $a(T_2)_0=9$ .

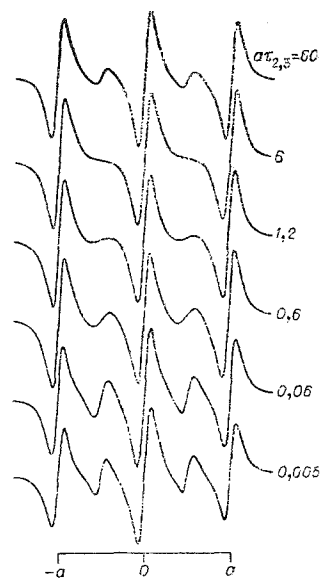


Fig. 8

Fig. 8. Influence of the rate of motion within the cage on the form of the ESR spectra of a biradical with three conformations:  $P_1=0.5$ ,  $P_2=P_3=0.25$ ,  $a\tau_1=60$ ,  $\tau_2=\tau_3$ ,  $J_1=J_2=0$ ,  $J_3=300a$ ,  $a(T_2)_0=9$ .

In the case where  $|J| \gg a$ , the alternated broadening of the observed T lines is described by the expression [4, 47]

$$\Delta(1/T_2) = a^2(m_1 - m_2)^2 j(\Delta\Omega)/4\bar{J}^2. \quad (23)$$

Equations (20)-(23) become unsuitable, however, when the condition of weakly dephasing exchange is no longer fulfilled. In this case, which is encountered frequently, Eqs. (20)-(23) decrease considerably the width of the broadened ESR lines.

The alternated broadening of the lines in the general case apparently cannot be expressed analytically; moreover, an exact analytical expression of this kind may also prove to be useless for the interpretation of experimental spectra, in view of the need to take account of the intrinsic width of the components and the distortion produced by the superposition of different lines; all this makes it necessary to use the computer for the detailed analysis of the spectra. In this case it is undoubtedly more rational not to use analytical equations but to use the system of equations (15) directly. We shall nevertheless put forward some considerations which may prove to be useful in the semiquantitative analysis of alternated broadening in the case where  $|\bar{J}| \gg a$ .

Since dynamic electron spin exchange in biradicals is associated with conformational transitions, it is possible in the calculation of the linewidths to use the analogy between chemical and dynamic spin exchange. This analogy is not obvious, in spite of the fact that the transition from one conformation to another is ordinary chemical exchange. There is considerable misunderstanding of this question in the literature devoted to nitroxide biradicals, so that we shall consider this problem in some detail (see also [18]).

In the case of chemical exchange, when one spin jumps between two states and the difference in the Zeeman frequencies is  $\Delta\omega$ , while the average lifetimes of the states are  $\tau_1$  and  $\tau_2$  respectively (Fig. 6), the form of the ESR spectrum is effectively described by modified Bloch equations [52, 53]. This is a classical system and it is well known [52] that for this system the condition for averaging of the two components of the spectrum, that is, the condition for rapid exchange, is given by

$$(\Delta\omega)^2[\tau_1\tau_2/(\tau_1 + \tau_2)]^2 \ll 1, \quad (24)$$

and the broadening of the Lorentzian line obtained on averaging is equal to

$$\Delta(1/T_2)_{\text{chem. exch.}} = (\Delta\omega)^2\tau_1^2\tau_2^2/(\tau_1 + \tau_2)^3. \quad (25)$$

In the biradical, however, we have a case of spin exchange, involving two spins simultaneously. We can consider the previously examined simplest model of a hypothetical biradical with two conformations and with spin resonance frequencies  $\omega_1$  and  $\omega_2$ . For simplicity we shall assume that  $|J_1| \ll \Delta\omega$ ,  $|J_2| \gg \Delta\omega$ , and  $\tau_2 \ll \tau_1$  (see Fig. 6). We shall also assume that the exchange integral is sufficiently large for significant changes in the phase of the precession of the spins to take place in a time interval  $\tau_2$  (in other words the condition for strongly dephasing spin exchange is fulfilled  $(J_2\tau_2)^2 \geq 1$ ). Using the language of chemical exchange for the description of spin exchange in the biradical, we should say that the process determining the form of the spectrum is the exchange of the resonance frequencies  $\omega_1$  and  $\omega_2$  by means of the exchange interaction  $J_2$  in a time  $\tau_2$ . It can be seen from Fig. 6 that in this case the picture of spin exchange coincides with the picture of chemical exchange, but the transitions take place between states with equal lifetimes. Both of these times are equal to  $\tau_1/F$ ,

where  $F \leq 1/2$  is determined by the dephasing "strength" of the spin exchange and is given by  $F = \frac{(J_2\tau_2)^2}{2\{1+(J_2\tau_2)^2\}}$  (see for example [6, 54, 55]). For  $(J_2\tau_2)^2 \gg 1$ ,  $F \approx 1/2$ . From this it can be seen that in the case of strongly dephasing spin exchange being discussed, the condition for rapid exchange is the inequality  $\Delta\omega^2\tau_1^2 \ll 1$  (or in more general form  $\Delta\omega \cdot \max\{\tau_1, \tau_2\} \ll 1$ ), and not the condition  $\Delta\omega \cdot \min\{\tau_1, \tau_2\} \ll 1$ , as follows from Eq. (24).

Here, the broadening of the lines  $\Delta(1/T_2)$ , calculated by means of Eq. (25) for chemical exchange, is found to be equal to

$$\Delta(1/T_2)_{\text{spin exch.}} = (\Delta\omega)^2\tau_1/8F. \quad (27)$$

More rigorous calculation using the dynamic equations for the spin density matrix leads to the same result. Equation (27) is applicable for  $|\bar{J}| \gg \Delta\omega$  for both strongly and weakly dephasing exchange. In the case where  $\tau_2 \geq \tau_1$ , simple expressions of this kind could not be obtained for  $\Delta(1/T_2)$ , but numerical calculations using Eqs. (15) show [18, 45] that in this case Eq. (27) apparently still remains fully applicable.

From this it may be concluded that the alternated broadening  $\Delta(1/T_2)$  of the ESR lines of real biradicals is conveniently described (at least for  $|\bar{J}| \gg a$ ) by means of an effective value  $\tau_{\text{eff}}$ , which has the sense of the time between two successive exchange interactions:

$$\Delta(1/T_2) = (\Delta\omega)^2\tau_{\text{eff}}/4. \quad (28)$$

In the case where the biradical has a large ( $N \geq 3$ ) number of conformations, the classification of the spectra developed for the simplest model of the biradical cannot be applied directly. This is due to the fact that to determine the rate of exchange it is now necessary to take account of not only the values of  $\tau_j$  but also the values of the probabilities of the transition  $p_{jl}$  into other conformations. This may lead to a situation in which it is possible to divide all the conformations of the biradical into separate groups, within which rapid (with frequencies  $\nu_{jl} \equiv p_{jl}/\tau_j \geq \Delta\omega$ ) averaging motion takes place, while the transitions between different groups will correspond to the conditions of slow exchange ( $\nu_{jl} < \Delta\omega$ ). In this case the overall ESR spectrum of the biradical is roughly the superposition of the ESR spectra of separate groups of conformations with the properties indicated. The position of the ESR lines of each group of conformations with rapid exchange within the group can be described, as in the case of the two-conformation model, by the averaged exchange integral

$$\bar{J} = \Sigma' J_j p_j / \Sigma' p_j, \quad (29)$$

where the prime denotes summation over all conformations of the group with rapid exchange within the group. It should be noted that the averaging in Eq. (29) is carried out with respect to the relative concentrations of the conformations, and not with respect to their lifetimes, as is frequently postulated in the literature [47, 48].

As an illustration, Figs. 7 and 8 give the spectra of dynamic biradicals with two or with three conformations, calculated on a computer using Eqs. (15).

## 2. Interpretation of the Experimental ESR Spectra of Liquid Solutions of Biradicals. Effective Conformations

The difficulty of interpreting the isotropic ESR spectra of liquid solutions of biradicals is associated chiefly with two features.

Firstly, it is not known beforehand whether the spectrum is the superposition of the spectra of different conformations or whether it represents a picture averaged by rapid intramolecular motion. Simple inspection of the spectrum is often insufficient to resolve this problem, since, as noted above and as can be seen from Figs. 7 and 8, the qualitative form of the ESR spectra may be the same for cases of rapid and slow exchange.

Secondly, it is generally not known how many conformations the biradical possesses, and what should be an accurate dynamic model. It is known that even for comparatively simple nitroxide biradicals it is possible to distinguish six or more conformations [30]. Moreover, the marked orientational dependence of the magnitude of the exchange interactions [56] leads to a situation in which even rotational isomers [57] of the molecule with identical distances between the N-O fragments can be regarded as different conformations from the ESR viewpoint.

The first of these problems can in principle always be resolved. In fact, a characteristic feature of rapid exchange is that the ESR spectrum consists of alternately broadened lines, the position of which is described by the averaged value of the exchange integral  $\bar{J}$ . By changing the conditions of the experiment, for example by changing the temperature of the specimen, it is possible to produce a significant change in the averaged value  $\bar{J}$ , that is, to change the positions of the biradical components of the spectrum. In the case of slow exchange (and naturally in the case where there are no side factors such as the formation of chemical compounds by the molecules of the biradical and the solvent), change in temperature should not influence the position of the biradical lines. Moreover, the superposition of spectra, that is, the existence of slow exchange, can be detected from the deviation of the integral intensity of the components from the relationship given by Eqs. (6), and also from the presence in the spectra of a larger number of lines than predicted by Eqs. (5). Thus a correctly designed experiment will give a sufficiently unambiguous answer to the question of whether rapid or slow exchange is taking place.

The problem of the adequacy of the dynamic model is much more complicated. This problem can apparently be resolved (more or less completely) only within the framework of the concept of the effective conformations of the biradical.

The basic idea of the method of effective conformations is as follows [18, 58]. We assume in the simplest case that the complete set of conformations of the real molecule of the biradical can be divided into two groups  $\{1a, 1b, \dots, 1n_1\}$  and  $\{2a, 2b, \dots, 2n_2\}$  (see Fig. 9), and also that:

a) the exchange integrals in each group have similar values, for example

$$|J_{j_1}| \ll a \text{ and } J_{j_2} \approx J_2, \tag{30}$$

b) the frequencies of the conformational transitions  $\nu_{\text{int}}$  within each group are much greater than the frequency of the transitions between conformations from different groups, that is, for the energies of activation of the transitions the following relationship applies:

$$\varepsilon_1, \varepsilon_2 \ll \varepsilon \tag{31}$$

(see Fig. 9).

If in addition

$$\nu_{\text{int}} \gg a, \tag{32}$$

it is impossible to determine by the ESR method how many conformations (one or more) are in fact present in each group, since, as indicated above (p. 110), with condition (32) each group will behave as a single effective conformation (denoted "1" and "2" respectively). Conditions (30) and (31) also make the conformations within each group thermodynamically indistinguishable (naturally, within a definite temperature range).

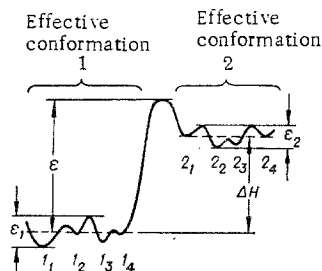


Fig. 9. Determination of the effective conformation.

Each of the two effective conformations can be assigned its own lifetime  $\tau_j$ , and when condition (31) is fulfilled the ratio  $\tau_2/\tau_1$  is described by the Arrhenius equation

$$\tau_2/\tau_1 = \exp \{ \Delta S/R - \Delta H/RT \}. \quad (33)$$

Here,  $\Delta S$  and  $\Delta H$  are constants having the significance of the difference in the entropies and enthalpies of the effective conformations. With condition (31)

$$\Delta H \approx \sum_{j=1}^{n_2} H_{2j} P_{2j} - \sum_{l=1}^{n_1} H_{1l} P_{1l} \quad (34)$$

and

$$\Delta S \approx \sum_{j=1}^{n_2} S_{2j} P_{2j} - \sum_{l=1}^{n_1} S_{1l} P_{1l}, \quad (35)$$

where  $S$  and  $H$  are the entropies and enthalpies of the real conformations, and  $P$  represents the normalized relative concentrations in each group;

$$\sum_{l=1}^{n_1} P_{1l} = \sum_{j=1}^{n_2} P_{2j} = 1$$

(compare with the averaging of Eq. (29)).

For the lifetime  $\tau_1$  the following relationship is fulfilled:

$$\tau_1 = \tau_0 \exp \{ \epsilon/RT \}, \quad (36)$$

where  $\tau_0$  is the characteristic time for the motion by means of which a transition would take place between the effective conformations in the absence of an energy barrier between them.

As a consequence of condition (30), each effective conformation is described by the exchange integrals

$$J_1 = \sum_{l=1}^{n_1} J_{1l} P_{1l} \quad \text{and} \quad J_2 = \sum_{j=1}^{n_2} J_{2j} P_{2j},$$

which are independent of temperature.

A noteworthy feature is that conditions (30)-(32) are necessary and sufficient for the realization of the Arrhenius relationships (33) and (36). Thus if the form of the ESR spectrum of a real biradical is described sufficiently effectively by the system of Eqs. (15) for the two-conformation model, then the experimentally obtained Arrhenius relationship (33) for the ratio  $\tau_2/\tau_1$  will provide a strong argument for the applicability of the two-conformation model.

This relationship in the case of slow exchange is determined from the statistical weights of the spectra of the different effective conformations (by means of Eqs. (6)), and in the case of rapid exchange it is determined from the broadening of the biradical components of the spectrum or the displacement of the lines as a consequence of change in the measured average exchange integral  $\bar{J}$  (19).

The concept of effective conformations is naturally also applicable in the case where the ESR spectrum of a real biradical cannot be described by the simplest two-conformation model. In this case the term effective conformation is used to describe that group of real conformations of the biradical for which conditions of types (30)-(32) will be applicable. The effective conformation "j" is characterized by the exchange integral  $J_j$ , the average lifetime  $\tau_j$ , and the relative concentration  $P_j$  ( $\sum P_j = 1$ ) (compare with the previous section).

The following procedure can be proposed for the experimental separation of the individual effective conformations [18, 58]: In the ESR spectrum of the biradical it is necessary to distinguish groups of lines, the positions of which are described by Eqs. (5). As already noted, the presence of several such groups in the spectrum indicates the superposition of spectra, that is, that there is slow motion (for nitroxide biradicals  $\nu < a \sim 3 \cdot 10^8$  rad/sec  $\approx 5 \cdot 10^7$  Hz) in the biradical.

If with change in temperature the position of the lines of any group remains unchanged, and the width is the same for all lines of this group and the relative intensities of the lines are described by Eqs. (6), this group of lines must be assigned to a single effective conformation with a corresponding exchange integral. As a consequence of condition (30), this integral characterizes sufficiently effectively the corresponding real conformations of the biradical. We cannot exclude the possibility that superpositions of the spectra of effective conformations with identical  $J_j$  may be observed (the probability of this situation is particularly high for  $|\bar{J}_j| \ll$

$a$  or  $|J_j| \gg a$ ). These conformations can be distinguished only by carefully verifying the applicability of relationships of types (33) and (36) between the experimentally determined  $\tau_j$  and  $P_j$ .

If the position or width of the lines changes with change in temperature, the effect of rapid intramolecular motion is being observed, and the given group of lines corresponds to not one but two or more effective conformations. To determine the true number of effective conformations taking part in rapid exchange, it is again necessary to carry out a detailed analysis of the displacement or broadening of the lines with change in temperature and to study the applicability of Eqs. (33) and (36).

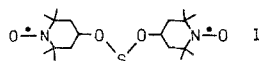
To conclude this section it may be noted that the study of biradicals by the ESR method loses sight of conformations with a small statistical weight and with any value of  $J$ , if they are not taking part in rapid exchange. In the case where conformations of this kind are involved in rapid exchange, even a low concentration of these conformations may produce an appreciable influence on the values of, for example,  $\bar{J}$  and  $\Delta(1/T_2)$ . Thus from the viewpoint of the study of the intramolecular dynamics of biradicals or the use of biradicals as SP, the use of biradicals with rapid exchange is generally preferable. For this purpose it is particularly convenient to use biradicals with  $|\bar{J}| \sim a$  and rapid exchange – the simultaneous study of the change in the position and width of the biradical components of the spectrum can give the most complete information on the state of the molecule of a biradical in solution.

### 3. Study of Intramolecular Motion in Biradicals

In the present section we examine specific examples of the analysis of the dynamics of the intramolecular motion of nitroxide biradicals.

#### a. Examples of Rapid and Slow Exchange in Biradicals

The spectra of a toluene solution of biradical I, recorded at different temperatures [59], are given in Fig. 10. It can readily be seen that the entire set of biradical lines "1," "2," and "3" of each spectrum in Fig. 10 is effectively described by Eqs. (5) with a single value of  $|J|$  (compare with Fig. 2). With change in temperature, however, the biradical lines are displaced (Fig. 10). As noted above, the explanation for this displacement of the lines is that the position of the components of the spectrum is determined by the averaged value of the exchange integral  $\bar{J}$ . Thus the biradicals I provide a typical example of a biradical with rapid exchange. The rapid ( $\nu > 5 \cdot 10^7$  Hz) intramolecular motion apparently corresponds to rotation about the S–O bond, since these rotations are not associated with a high activation energy.



It is reasonable to assume that fulfillment of the conditions of slow exchange can be detected in biradicals for which conformational transitions are hindered by high activation energies.

An example of a biradical of this kind is provided by the biradical II [59], which is similar to biradical I,

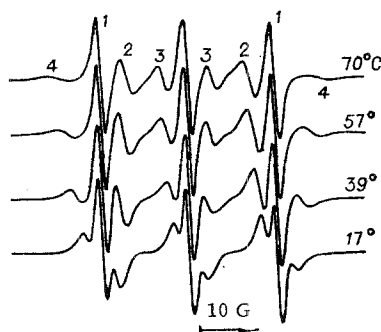
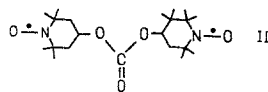


Fig. 10. ESR spectra of a toluene solution of biradical I.

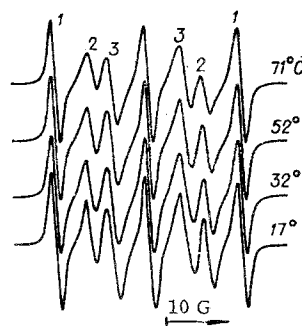


Fig. 11. ESR spectra of a toluene solution of biradical II.

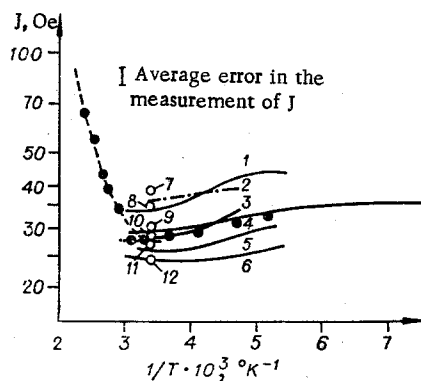


Fig. 12. Temperature dependence of the measured value of  $J$  for the biradical II in different solvents:  $-\cdot-\cdot-$ ) data [70] for  $\text{CHCl}_3$ ,  $---$ ) data [48] for: 1)  $\text{CHCl}_3$ , (for  $\text{CHBr}_3$  the values of  $J$  are the same [48]), 3) dimethylformamide (the points  $\bullet$  correspond to the data from [30] for the same solvent), 4)  $\text{CS}_2$  (for  $\text{CH}_3\text{I}$ ,  $\text{Si}(\text{CH}_3)_4$ ,  $\text{CH}_3\text{OH}$ ,  $\text{CH}_2\text{Cl}_2$ , and  $\text{CH}_3\text{CCl}_3$  the values of  $J$  are the same [48]), 5) isopentane, 6) n-hexane;  $\dots$ ) data [59, 61] for toluene, benzene,  $\text{CCl}_4$ , acetone, and  $\text{C}_2\text{H}_5\text{OH}$ ;  $\circ$ ) data [70] for: 7) water, 8)  $\text{CH}_3\text{COOH}$ , 9) acetone, 10) dimethylformamide, dimethyl sulfoxide, and  $\text{CH}_3\text{OH}$ , 11) xylene, 12) hexane. The broken part of curve 3 corresponds to measurements from the position of the T resonance lines 2 and 3 (see Fig. 11) without corrections for overlap of the lines.

and for which rotation about the C-O bond is hindered by the carbonyl oxygen atom. The changes in the ESR spectra of a toluene solution of biradical II with change in temperature are shown in Fig. 11. It can be seen from Fig. 11 that the position of the biradical lines is again effectively described by Eqs. (5) for  $|J| = 1.8a$ . In contrast to the situation for biradical I, however, for biradical II the position of the biradical lines "2" and "3" is practically independent of temperature (see Fig. 12).<sup>†</sup> Moreover, it can be seen that with increase in temperature the relative intensities of the lines "2" and "3" decrease compared with the intensity of the basic lines "1." The decrease in the intensities of the lines "2" cannot be explained on the basis of alternated broadening of the components of the spectrum, since in the temperature range 273-338°K the ratio of the intensities of the lines "1" and "2" changes by a factor of almost two (Fig. 11) while the widths of these lines remain constant (within the limits of accuracy of the measurements). Thus in the ESR spectra of biradical II, superposition of the spectra of different conformations is observed, and this is an indication of slow ( $\nu < 5 \cdot 10^7$  Hz) intramolecular motion in biradical II. Here, the value of  $|J| \approx 1.8a$ , which is measured from the spectra and which is independent of temperature, is a parameter characterizing the effective (in the sense of conditions (30)-(32)) conformation of this biradical.

Analysis of the temperature dependence of the value of  $\bar{J}$  for the biradical I and the ratio of the intensity of the biradical component "2" to the intensity of the basic component "1" for biradical II makes it possible to reach certain conclusions regarding the relative thermodynamics of the conformations in these biradicals [18, 61].

In fact, for biradical I, with decrease in temperature  $|\bar{J}| \rightarrow 0$ , indicating the existence of conformation "1" with  $|J_1| \ll a$ . Since there are grounds for assuming the existence, in this biradical, of another conformation "2" with  $|J_2| \approx (7.4 \pm 0.5)a$  (see below, p. 137), Eqs. (19) and (33) can be used within the framework of the

<sup>†</sup> The reported [38, 48] significant increase in  $J$  for biradical II when the specimens are heated above 60°C (see Fig. 12) is probably not real. This may be due to the fact that at sufficiently low temperatures the measurement was carried out from the position of outer satellites (S lines), which make it possible to measure  $J$  with a high accuracy. The rapid decrease in the intensity of the S lines (corresponding to the decrease in the intensity of the T lines) with increase in temperature makes it necessary to use for the measurement of  $J$  the position of the lines "2" (T lines). As a consequence of the poor resolution of the spectrum and the low intensity of the lines "2" at  $t > 60^\circ\text{C}$ , the values of  $|J|$  calculated from the observed position of the lines "2" are found to be appreciably too high, without an appropriate correction for the overlap of the components of the spectrum (see for example [60]).

two-conformation model to find the ratio  $\tau_2/\tau_1$  and obtain the estimate  $\Delta H = (3.0 \pm 0.1)$  kcal/mole and  $\Delta S = (-0.2 \pm 0.1)$  eu. It should be noted that the ratio  $\tau_2/\tau_1$  is given by a straight line on Arrhenius coordinates, and this apparently indicates that the two-conformation model is applicable to the description of the change in biradical I. Thus for biradical I the difference in the lifetimes of the conformations is determined practically entirely by the difference in their enthalpies, and the thermodynamically most favorable conformation is that with the small  $|J|$ . Assuming  $\tau_0 \sim 10^{-11}$  sec, we obtain from condition (18) for rapid exchange the estimate  $\varepsilon < 6$  kcal/mole.

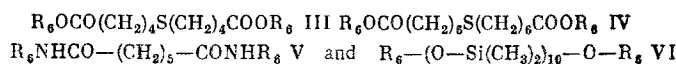
For biradical II, again assuming the existence of two conformations - "1" with  $J_1=0$  and "2" with  $|J_2| = 1.8a$  - and using Eqs. (6), we can readily determine the change in the statistical weights of the different conformations with change in temperature and hence find the temperature dependence of  $\tau_2/\tau_1$ . This relationship is also given by a straight line on Arrhenius coordinates, and  $\Delta H = (-3.1 \pm 0.1)$  kcal/mole and  $\Delta S = (8 \pm 1)$  eu. From condition (17) for slow exchange, the activation energy  $\varepsilon$  for the conformational transitions in this biradical can be estimated as  $\varepsilon > 4$  kcal/mole. Thus for biradical II the energetically most favorable conformation is that with high  $|J|$ , but it is not favored by entropy.

### b. Mechanism of Spin Exchange in Long-Chain Biradicals

The establishment of the fact of rapid exchange in biradical I was favored by the fact that for this biradical  $|J| \sim a$ , and change in temperature is accompanied by a marked displacement of the biradical components of the spectrum. Unfortunately, for a considerable number of biradicals which have been synthesized, the observed ESR spectra correspond to the case of very strong exchange ( $|J| \gg a$ ), and this excludes the possibility of observing an appreciable displacement of the lines in the case of rapid exchange (see Fig. 2).

These characteristic five-line spectra (Fig. 13) are typical of nitroxide biradicals with a long chain (8-10 or more  $-\text{CH}_2-$  fragments), in which exchange interactions between the radical centers of the molecule take place by direct collisions of the N-O fragments [4, 14, 62-67] (the values of the exchange integral  $|J| \sim 10^{10}$ - $10^{11}$  rad/sec [24-27, 38-43]).

A detailed analysis has been carried out [68] for the ESR spectra of the long-chain biradicals



(Here and subsequently,  $\text{R}_6 = \text{---} \text{N}^{\bullet} \text{O}$ ),

The marked decrease in the width of the biradical lines 2 and 2' for these biradicals with increase in temperature (see Fig. 13) indicates unambiguously that there is rapid modulation of the exchange interaction by intramolecular motion. At temperatures when the width of the lines 2 and 2' is close to the width of the lines 1 and 1', however, the distribution of the line intensities in the spectrum differs appreciably from the ratio 1:2:3:2:1 (see Fig. 13), predicted by the model of the biradical with two conformations and with strong rapid exchange. The measured ratio of the integral intensities indicates the superposition of the spectra of different conformations, that is, slow exchange.

Thus for a sufficiently correct interpretation of intramolecular motion in long-chain biradicals it is necessary to take account of at least three different conformations.

In fact, we can assume that there exists an "elongated" conformation "1," in which  $J_1=0$ , and that there also exist conformations "2" and "3," in which the radical fragments approach one another, and that in confor-

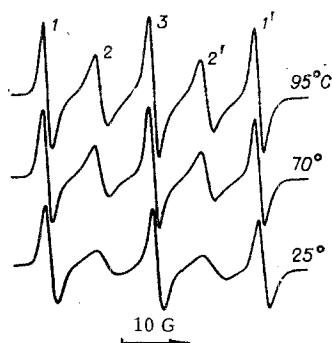


Fig. 13. Temperature dependence of the form of the ESR spectrum of a dimethyl sulfoxide solution of the long-chain biradical VI.



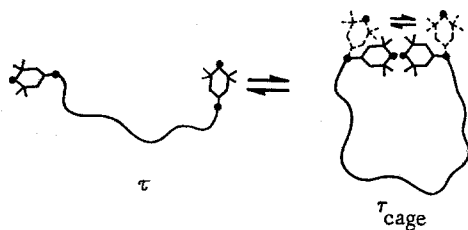


Fig. 14

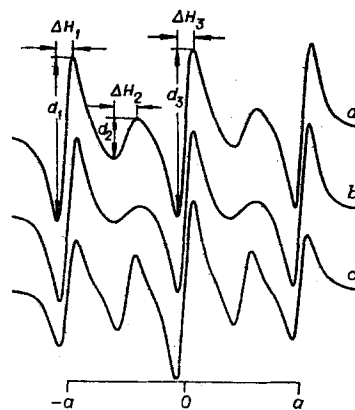


Fig. 15

Fig. 14. Diagram of the proposed "cage" mechanism of exchange interactions in long-chain nitroxide biradicals.

Fig. 15. Dependence of the values of  $(\tau_{\text{cage}}/\tau)$  and  $a\tau_{\text{eff}}$ , calculated from Eqs. (40)-(42), on the form of the ESR spectra of biradicals with three conformations. For all the spectra  $P_1=1/3$  ( $(\tau_{\text{cage}}/\tau)_{\text{theor}}=2$ ),  $a\tau_1=60$ ,  $J_1=J_2=0$ ,  $J_3=300a$ ,  $a(T_2)_0=9$ . a)  $P_2=1/3$ ,  $a\tau_2=0.6$ ,  $a\tau_3=0.6$ ,  $(\tau_{\text{cage}}/\tau)_{\text{expt}}=0.6$ ,  $a\tau_{\text{eff}}=0.31$ ; b)  $P_2=0.6$ ,  $a\tau_2=0.6$ ,  $a\tau_3=0.0667$ ,  $(\tau_{\text{cage}}/\tau)_{\text{expt}}=0.55$ ,  $a\tau_{\text{eff}}=0.35$ ; c)  $P_2=0.0667$ ,  $a\tau_2=0.0667$ ,  $a\tau_3=0.6$ ,  $(\tau_{\text{cage}}/\tau)_{\text{expt}}=2.0$ ,  $a\tau_{\text{eff}}=0.04$ .

mation "2,"  $J_2=0$ , and that in conformation "3,"  $|J_3| \gg a$  (Fig. 14). In other words, it is assumed that when they approach one another, the radical fragments enter a common "cage," in which they may or may not interact. If the lifetime of the radical fragments is  $\tau_{\text{cage}}$  in the cage, and  $\tau$  outside the cage, it follows that for sufficiently long lifetimes  $\tau_{\text{cage}}$  and  $\tau(a \cdot \max\{\tau_{\text{cage}}, \tau\} > 1)$  superposition of the ESR spectrum of the cage and the ESR spectra of the radicals outside the cage will be observed (with statistical weights  $\tau_{\text{cage}}/(\tau_{\text{cage}} + \tau)$  and  $\tau/(\tau_{\text{cage}} + \tau)$  respectively). Here, in the case of rapid motion of the radical fragments within the cage the ESR spectrum of the cage is described by the model of rapid exchange.

Thus the change in the width of the lines 2 and 2' must be attributed to changes in the conditions of modulation of the exchange integral only within the cage, whereas the observed ratio of the intensities of the lines is due to the superposition of the ESR spectra of the cage and the radicals outside the cage.

The fact that the width of the lines 2 and 2' is due entirely to motion within the cage is confirmed by the fact that in identical solvents in biradicals with similar structures the width of the lines 2 and 2' depends only on temperature, but not on the chain length [68]. At the same time the relative intensity of these lines decreases with increase in the chain length, since there is a decrease in the ratio  $\tau_{\text{cage}}/\tau$ , and hence in the statistical weight of the spectrum of the cage.

The applicability of the qualitative cage model for the interpretation of the ESR spectra of long-chain biradicals is confirmed by analysis of the form of the spectrum of the systematic three-conformation model of the biradical [18, 45].

In fact, if for the state "1" of the biradical outside the cage the inequality  $a\tau_1 \gg 1$  is fulfilled, then in the case of rapid motion within the cage,  $a\tau_2, a\tau_3 < 1$  and

$$\tau_{\text{cage}} = \tau_1 \frac{P_2 + P_3}{P_1} = \tau_1 \frac{1 - P_1}{P_1}. \quad (37)$$

For those biradicals for which biradical lines are observed,  $P_2 + P_3 \sim P_1$ , and, since  $\tau_1 \gg \tau_2, \tau_3$ , Eq. (11) leads necessarily to the relationship

$$P_2/\tau_2 \approx P_3/\tau_3. \quad (38)$$

Thus the dynamics of intramolecular motion in the proposed model of long-chain biradicals are described in principle by four independent parameters, for example

$$\tau_1, \tau_2, \tau_3 \text{ and } P_1. \quad (39)$$

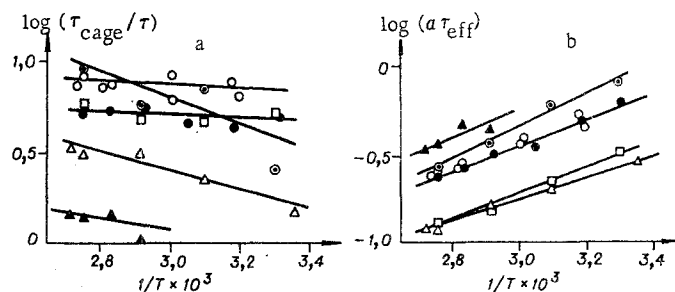


Fig. 16. Temperature dependence of the observed values of: a)  $\tau_{\text{cage}}/\tau$  and b)  $a\tau_{\text{eff}}$  for biradicals III-VI.  $\odot$ ) Biradical III in DMSO,  $\circ$ ) III in benzene,  $\bullet$ ) III in toluene,  $\blacktriangle$ ) IV in DMSO,  $\square$ ) V in benzene,  $\triangle$ ) VI in DMSO.

Here, as expected, the spectra obtained by calculation using Eqs. (14) and (15) give all the characteristic features of the experimental ESR spectra of long-chain nitroxide biradicals. For example, Fig. 8 demonstrates the influence of the rate of exchange within the cage on the form of the overall ESR spectrum.

Detailed analysis [45] of the possibility of a complete interpretation of the experimental ESR spectra of long-chain biradicals showed that, unfortunately, it is impossible to determine all the parameters of the intramolecular dynamics and exchange directly from the ESR spectra, since in addition to the parameters (39) the ESR spectrum is also described by the intrinsic width  $(1/T_2)_0$  of the ESR lines  $((1/T_2)_0 = \sqrt{3/2} \cdot \Delta H_1$  - see Fig. 15), and by the value of the exchange integral  $J_3$ , which is not known a priori. Moreover, the calculations show that for sufficiently rapid motion within the cage the characteristic features of the motion within the cage (for example long and short duration of exchange interactions) cannot be determined in principle (see Fig. 15), and the interpretation of the experimental spectra is most complete within the framework of the cage model.

Since the cage is described by one averaged conformation with strong rapid exchange (that is  $|\bar{J}| \gg a$ ), the experimental ESR spectra can readily be used to find the ratio  $\tau_{\text{cage}}/\tau$ , by comparing the integral intensities  $I_1$  and  $I_2$  of the lines 1,1' and 2,2' respectively. The order of magnitude of the ratio of  $I_1$  to  $I_2$  can be estimated from the simple relationship

$$\frac{I_1}{I_2} \approx \frac{d_1 (\Delta H_1)^2}{d_2 (\Delta H_2)^2} \quad (40)$$

(the symbols are defined in Fig. 15). Since the observed spectrum is the superposition of two spectra (triplet and quintet) we obtain

$$\frac{\tau_{\text{cage}}}{\tau} = \frac{3I_2/I_1}{2 - I_2/I_1} \quad (41)$$

Numerical calculations show (see Fig. 15) that for a width of the biradical components "2" close to the width of the basic components "1," the calculation of  $\tau_{\text{cage}}/\tau$  from Eqs. (40) and (41) gives a sufficiently accurate result, and that even in the case of extensively broadened biradical lines these equations give the correct (although slightly high) order of magnitude of  $\tau_{\text{cage}}/\tau$ . For a more accurate determination of the values of  $\tau_{\text{cage}}/\tau$  it is necessary to use numerical modeling of the alternately broadened spectrum of the biradical, for example with the assumption that we are observing the superposition of two spectra with lines of Lorentzian form, broadened in accordance with Eq. (28).

TABLE 1. Parameters  $\Delta H$ ,  $\Delta S$ ,  $\tau_0$ , and  $\epsilon$  for the Long-Chain Biradicals III-VI (from the data in [68]).

Biradical	Solvent	$\Delta H$ , kcal/mole	$\Delta S$ , eu	$-\log(\tau_0) \pm 1$ , $\tau_0$ , sec	$\epsilon \pm 0.6$ , kcal/mole
III	Benzene	$<0.5$	$4.1 \pm 2$	11.5	3.2
III	Toluene	$<0.5$	$2.9 \pm 2$	11.7	3.4
III	Dimethyl sulfoxide (DMSO)	$2.7 \pm 1.5$	$11.8 \pm 4$	12.2	4.2
IV	DMSO	$1.3 \pm 1$	$2.0 \pm 2$	11.7	3.7
V	Benzene	$<0.5$	$2.9 \pm 2$	12.1	3.5
VI	DMSO	$2.4 \pm 0.5$	$9.0 \pm 2$	11.7	2.9

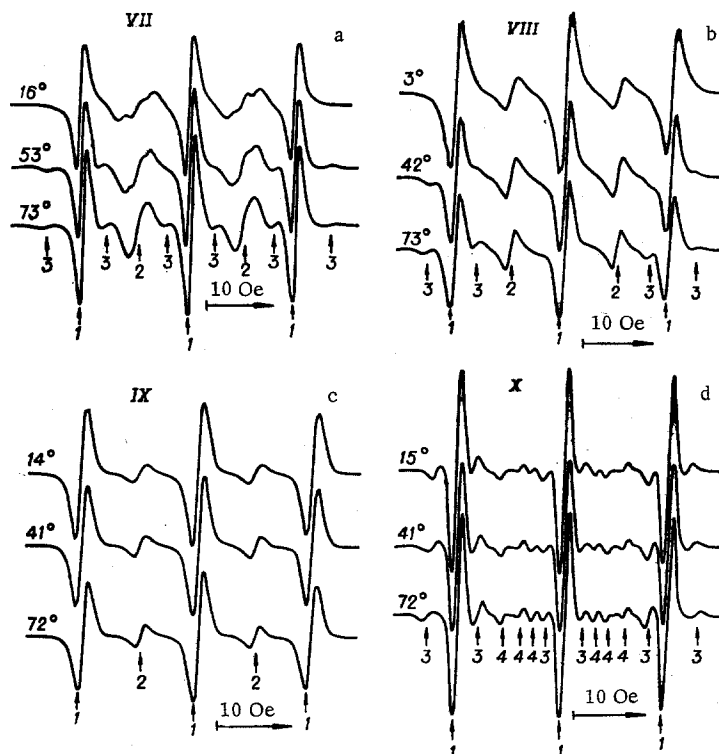


Fig. 17. ESR spectra of toluene solutions of the biradicals VII-X.

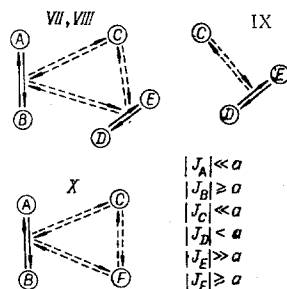


Fig. 18. Schematic representation of rapid (—) and slow (----) conformational transitions detected in biradicals VII-X.

TABLE 2. Characteristics of the Transitions between the Conformations for the Biradicals VII-X (from the data in [58])

Biradical	$\Delta H$ , kcal/mole	$\frac{\Delta S^\ddagger}{0.5}$ , eu	$\epsilon$ , kcal/mole	$-\log(\tau_0)$ , sec
VII	—	—	$2 \pm 0.2$	10,7
VIII	$0,6 \pm 0,1$	1,2	$4,8 \pm 0,8$	13,2
IX	$0,5 \pm 0,1$	-1,0	$> 5$	—
X	$2,3 \pm 0,3$	1,1	—	—

Using the concept of effective time  $\tau_{\text{eff}}$  (see Eq. (28)) of the modulation of the exchange interaction, it is possible to reach certain conclusions regarding the motion of the radical fragments within the cage.

For the cage model being examined,  $\tau_{\text{eff}}$  is a complex combination of the parameters  $\tau_2$ ,  $\tau_3$ , and  $J_3$ , and its significance corresponds to that of the time between two successive exchange interactions within the cage. With allowance for Eq. (28) and the fact that the form of the lines is close to Lorentzian, we obtain

$$a\tau_{\text{eff}} = 3.46(\Delta H_2 - \Delta H_1)/a. \quad (42)$$

The values of the quantities  $\tau_{\text{cage}}/\tau$  and  $a\tau_{\text{eff}}$  for the biradicals III-VI, calculated from Eqs. (40)-(42), are given in Fig. 16a on Arrhenius coordinates. An interesting feature is that, as expected,  $\tau_{\text{cage}}/\tau$  depends very little on temperature, since for long biradicals, entry into the cage is determined chiefly by steric factors (that is, entropy factors), and not energy factors.

Table 1 gives a summary of the entropy  $\Delta S$  and enthalpy  $\Delta H$  of the cage, found from Fig. 16, and also the parameters  $\tau_0$  and  $\varepsilon$ , describing the temperature dependence of  $\tau_{\text{cage}}/\tau$  and  $\tau_{\text{eff}}$  with the assumption that

$$\tau_{\text{cage}}/\tau = \exp\{\Delta S/R - \Delta H/RT\}$$

and

$$\tau_{\text{eff}} = \tau_0 \exp\{\varepsilon/RT\}$$

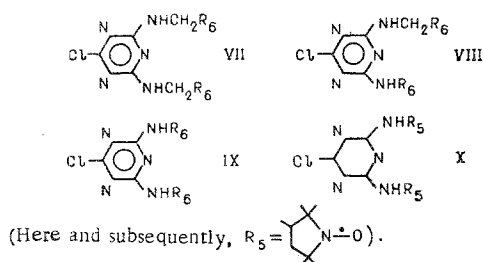
(compare with Eqs. (33)-(36) for "real" effective conformations).

An interesting feature is that the observed values of  $\tau_0$  for the biradicals III-VI are practically independent of the nature of the solvent and are equal to  $\sim 10^{-11}$  sec, characteristic of the rotation about simple  $\sigma$  bonds.

### c. Study of Complex Conformational Equilibria in Solutions of Nitroxide Biradicals

The above examples of biradicals in which the intramolecular motion observed by the ESR method is described sufficiently accurately by the two- or three-conformation model are the most typical. Nevertheless, there also exist nitroxide biradicals for which the description of the ESR spectra requires allowance for at least five different conformations.

Studies have been made [58] of the biradicals



The spectrum of biradical IX (Fig. 17c) consists of a quintet of lines "1" and "2," characteristic of the long-chain biradicals examined above. The broadening of the biradical lines observed with decrease in temperature, and also their low relative intensity, indicates the existence in this biradical of at least three different effective conformations: "C," "D," and "E," with  $|J_C| \ll a$ ,  $|J_D| \leq a$  and  $|J_E| \gg a$ , and that rapid transitions (with  $\nu > 5 \cdot 10^7$  Hz) take place between conformations "D" and "E," whereas conformation "C" undergoes only slow intramolecular motion.

The ESR spectra of biradicals VII and VIII (Fig. 17a and b), in addition to the groups of lines "1" and "2," also show other groups of lines "3." The position of these groups is described by Eqs. (5). Since the position of the lines "3" changes with change in temperature, these groups of lines must correspond to at least two conformations "A" and "B" ( $|J_A| \ll a$  and  $|J_B| > 0.4a$ ) with rapid transitions between them. Thus for the description of the ESR spectra of liquid solutions of biradicals VII and VIII it is necessary to take into account at least five different conformations.

The ESR spectra of the biradical X differs from the spectra of biradicals VII and VIII only in that in place of the lines "2" there is observed a group of lines "4" (Fig. 17d), the position of which does not change with change in temperature, that is, the lines "4" correspond to one effective conformation "F" with  $|J_F| = (3.3 \pm$

0.2)a. The disproportionately high relative intensity of the lines "1" indicates the presence in the biradical of another effective conformation "C" with  $|J_C| \ll a$ , with slow transitions from it. Thus the ESR spectra of the biradical X are described by a model taking account of four effective conformations.

Diagrams of the observed conformational transitions in biradicals VII-X are given in Fig. 18. Various thermodynamic parameters of the observed conformational transitions have also been determined [58]. Equation (41) has been used to find the ratio of the total lifetime ( $\tau_{DE}$ ) of the biradicals in the conformations D and E to the lifetime ( $\tau_R$ ) of the biradical in all other conformations. Table 2 gives the values of  $\Delta H$  and  $\Delta S$  found from these ratios using Eqs. (33), and the parameters  $\epsilon$  and  $\tau_0$ , which were determined from Eqs. (36) and (42) and which describe rapid transitions between the conformations D and E.

The measured temperature dependence of the value of the exchange integral  $|\bar{J}|$ , characterizing rapid transitions between the conformations A and B, made it possible to estimate the difference in the enthalpies  $\Delta H_{AB}$  between these conformations. In fact, from Eqs. (19) and (33) with the condition  $|J_A| \ll a$  it follows that

$$\ln \left| \frac{\bar{J}(T)}{a} \right| = \frac{\Delta S_{AB}}{R} - \frac{\Delta H_{AB}}{RT} + \ln \left| \frac{J_B - \bar{J}(T)}{a} \right| \quad (43)$$

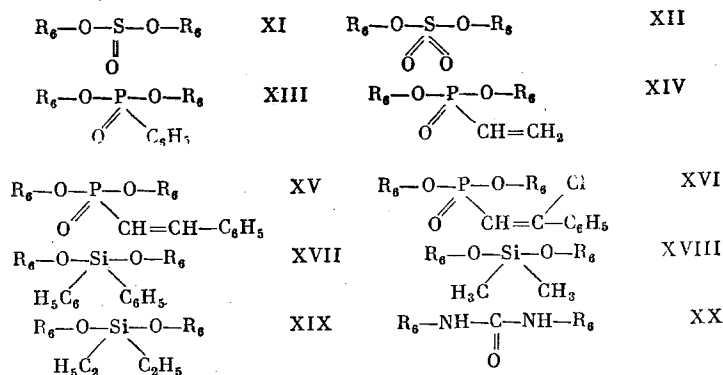
where  $\bar{J}(T)$  is the measured value of the averaged exchange integral, which depends on the temperature T. The value of  $J_B$  is unknown, so that it is possible to estimate approximately only the ratio of the values of  $\Delta H_{AB}$  for different biradicals, since the temperature dependence of  $\ln |\bar{J}/a|$  is determined chiefly by the enthalpy term of Eq. (43). It was found that  $\Delta H_{AB}(\text{VII}) \approx \Delta H_{AB}(\text{VIII}) \approx 2.4 \Delta H_{AB}(\text{X})$ , that is, the difference in the enthalpies of the conformations A and B is much greater for piperidine-oxyl biradicals than for pyrrolidine-oxyl biradicals.

#### 4. Mechanism of Exchange Interactions in Short Nitroxide Biradicals

An important stage in the study of biradicals is the investigation of the mechanism of the electron exchange interactions of the unpaired electrons. In principle, exchange interactions are short-lived, and it is often postulated that spin exchange requires the close approach (to  $\sim 3\text{-}5\text{\AA}$ ) of the N-O fragments. It is undoubtedly the case that for biradicals with widely separated N-O fragments (for example for the long-chain biradicals examined above), this approach is in fact necessary. As already noted, however, in addition to exchange by the direct overlap of the wavefunctions of the unpaired electrons, it is also possible in principle to have the participation, in the transfer of exchange interactions, of the atoms of the biradical itself ("indirect exchange mechanism") or a "bridge" of solvent molecules.

The possibility of the existence of an indirect mechanism of spin exchange in the molecules of nitroxide biradicals has been discussed practically since their original synthesis [4, 67]. Nevertheless, distinct experimental proof of the existence of indirect exchange was obtained only comparatively recently.

Studies have been made [18, 61] of a number of short biradicals of similar structure, in which piperidine rings are joined to one another by one C, S, Si, or P atom or two O (or N) atoms.



It was indicated above that the behavior of the ESR spectra of biradical II is described by the slow exchange model. For radicals XVII-XX it is known that the magnitude of the measured  $|J|$  is independent of temperature [69, 70]. For biradicals XI-XVI in the range 17-70°C, no appreciable change in the ESR spectra was observed [61]. The relative intensities of the biradical lines of their spectra coincide with the theoretical values, so that the biradicals XI-XVI are present in solution in practically only one conformation. Thus the values of  $|J|$  determined from the ESR spectra of biradicals II and XI-XX refer to definite effective conformations; these values of  $|J|$  are summarized in Table 3.

TABLE 3. Exchange Integrals at 25°C and Distances between the Iminoxyl Fragments at 77° for Toluene Solutions of the Biradicals I, II, XI-XX (from the data in [18, 61, 70])

Biradical	Connect- ing atom	$a \pm 0.2$ G	$ J/a $	$r \pm 0.6$ Å from the second moment	$r \pm 0.7$ Å from the dipole- dipole splitting	$r \pm 0.5$ Å from $\Delta M_S = 2$	$r_{\min} + r_{\max}$ Å
II	C	15,3	1,8	11,2	—	11,7	—
XX†	C	15,2	1,2	—	—	—	—
I	S	15,3	0,4‡	11,1	11,6	10,8	7÷15
XI	S	15,3	7,6	10,8	12,0	—	—
XII	S	15,3	7,1	10,7	11,9	10,3	8÷13
XIII	P	15,3	6,4	10,6	10,6	—	8÷13
XIV	P	15,4	6,5	10,3	10,9	10,2	8÷13
XV	P	15,3	6,7	10,7	10,7	—	8÷13
XVI	P	15,3	6,5	10,0	11,6	—	8÷13
XVII—							
XIX	Si	15,6	8-9	—	—	—	—

†In biradical XX the O atoms of the connecting bridge are replaced by NH groups. The data were taken from [70] for a solution in xylene and in acetone.

‡As a consequence of rapid exchange, the given value of J is in fact  $\bar{J}$ .

TABLE 4. Values of J and a, Measured for Biradicals II and XX in Various Solvents at 22°C

Solvent	Biradical II		Biradical XX		Literature cited
	J, G	a, G	J, G	a, G	
Isopentane	26				[48]
Hexane	24,3	15,2	15,8	15,2	[70]
	24,5				[48]
Benzene	28±1				[61], [59]
Toluene	28±1	15,3			[61], [59]
Xylene	27,1	15,2	18,6	15,2	[70]
CCl <sub>4</sub>	28±1				[61], [59]
CH <sub>2</sub> Cl <sub>2</sub>	29				[48]
CH <sub>3</sub> CCl <sub>3</sub>	29				[48]
CH <sub>3</sub> I	29				[48]
CS <sub>2</sub>	29				[48]
Dimethylformamide	28,9	16,0	18,7	15,8	[70]
	28				[30], [48]
Dimethyl sulfoxide	28,8	15,7	17,2	15,8	[70]
Acetone	30,3	15,6	18,2	15,5	[70]
	28±1				[59], [61]
C <sub>2</sub> H <sub>5</sub> OH	28±1				[61], [59]
CH <sub>3</sub> OH	28,7	16,0	17,4	16,1	[70]
	29				[48]
CH <sub>3</sub> COOH	34,7	16,2	20,6	16,4	[70]
CHCl <sub>3</sub>	35,6	15,8	24,2	15,9	[70]
	34				[48]
CHBr <sub>3</sub>	34				[48]
Water	38,6	17,0	23,4	17,1	[70]

A noteworthy feature is that for biradicals II and XI-XIX the magnitude of the exchange integral J for the effective conformation depends little on the nature of the substituents at the connecting atom (this makes possible the assumption, used above, that in biradical I there is an effective conformation with  $J_2$  close to the values  $|J| = (7.1-7.7)a$  for biradicals XI-XII). Moreover, for biradical II the magnitude of the exchange integral  $J_2$  depends very little on the nature of the solvent (see Fig. 12 and Table 4). For biradicals III-VIII also, J showed no appreciable dependence on the solvent.

An important characteristic of a conformation is the distance r between the N-O fragments. Table 3 summarizes the results of the measurement of this distance (see below) in toluene solutions vitrified at -196°C. It can be seen from Table 3 that the values of r are similar for all the biradicals discussed and are greater

than the minimum possible values  $r_{\min}$  (Table 3) obtained from an examination of Stuart-Briegleb molecular models.



The weak dependence of  $J$  on temperature and the solvent for biradicals II and XI-XX indicates that the measurements in liquid solutions give a static value of  $J$  corresponding to a definite conformation of the biradical. Moreover, the results of the direct interpretation of the spectra of the frozen solutions (see below) indicate that in vitreous toluene solutions of the biradicals II, XI, and XII the conformations stabilized are apparently those for which the values of  $J$  are measured in the liquid. Thus spin exchange in biradicals II and XI-XX takes place in the absence of direct "collisions" of the N-O groups, since the values of  $J$  refer to conformations with  $r > 10 \text{ \AA}$ .

In view of the very large distance ( $\sim 11-12 \text{ \AA}$ ) between the N-O fragments, spin exchange by direct overlap of the unpaired electron clouds is extremely unlikely. It is difficult to reconcile exchange through a "bridge" of solvent molecules [73, 74] with the weak dependence of  $J$  on the nature of the solvent. At the same time, none of the experimental data contradict a mechanism of exchange along the chain of  $\sigma$  bonds of the molecule of the biradical. This also explains the unambiguous dependence of the magnitude of  $J$  on the nature of the connecting atom. The fact that the values of  $|J|$  in the biradicals XI-XIX are greater than that in the biradical II can be attributed to the participation of the  $d$  orbitals of the Si, P, or S atoms in the delocalization of the unpaired electrons. Similar results for the comparative "electronic conductivity" of the C and Si atoms in the molecules of stable monoradicals were obtained by the NMR method [75]. An interesting feature is that the "electronic conductivity" of N atoms is lower than that of O atoms; this can be seen by comparing the values of  $J$  for the biradical II and the biradical XX [70], which differs from II only in the replacement of the O atoms of the connecting bridge (see Tables 3 and 4) by NH groups, and in which slow exchange is also observed.

If moreover it is assumed that a significant role is played by the hyperconjugation mechanism in the transfer of exchange interactions, it becomes possible to explain the dependence of the magnitude of  $J$  on the angle of rotation of the piperidine rings about the S-O or C-O bonds in the biradicals I and II.

It is necessary to emphasize specially the long-range action of the exchange interactions in biradicals I, II, and XI-XX. In spite of the fact that the unpaired electrons in these biradicals are separated by ten (!)  $\sigma$  bonds, the energy of the electronic exchange interactions along the chain of these bonds reaches  $4 \cdot 10^8 \text{ Hz}$ . From the analogy between electronic spin exchange and the process of electron transfer [74], in a number of

TABLE 5. Change in the Magnitude of the Exchange Integral with Change in the "Bridging" Groups Connecting the Radical Fragments (from the data in [18, 61, 69, 70, 79, 80])

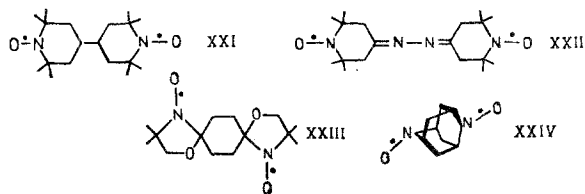
Connecting atom or group	Biradical	$ J/a ^\dagger$	reference atom or group	$\gamma$
Si	XVII	$8 \pm 1$	Si	1
C	II	$1,8 \pm 0,1$	Si	$4,5 \pm 0,5$
S	XI, XII	$7,4 \pm 0,3$	Si	$1,1 \pm 0,2$
P	XIII-XVI	$6,5 \pm 0,2$	Si	$1,2 \pm 0,2$
O	II	$1,8 \pm 0,1$	O	1
NH	XX	$1,15 \pm 0,1$	O	$1,12 \pm 0,1$
(-)	XXVII	$10 \pm 1$	(-)	1
(-)(CH=CH-)	-	-	(-)	$1,7 \pm 0,2^\ddagger$
(-)(CH=CH-) <sub>2</sub>	XXVIII	$3,1 \pm 0,2$	(-)	$3,2 \pm 0,5$
(-)(CH=CH-) <sub>3</sub>	XXIX	$1,8 \pm 0,1$	(-)	$5,6 \pm 1$
(-) (  )	XXX	$1,7 \pm 0,1$	(-)	$6 \pm 1$
(-) (  )	XXXI	$2,7 \pm 0,2$	(-)	$3,7 \pm 0,5$

<sup>†</sup>For the biradicals XXVII-XXXI the values of  $|J/a|$  relate to the averaged value of  $\bar{J}$  at  $20^\circ\text{C}$ .

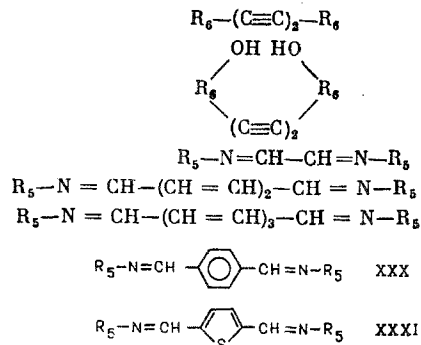
<sup>‡</sup>Obtained by comparing  $\gamma$  for the biradicals XXVII, XXVIII, and XXIX.

cases it is possible to expect an appreciable probability of electron transfer over large distances along a chain of  $\sigma$  bonds, and this may be significant for an understanding of a number of biochemical processes.

In addition to the biradicals I, II, and XI-XX, a number of shorter rigid biradicals are also known (for example [4, 14, 28, 29, 76])



in which exchange interactions undoubtedly take place along a chain of atoms in the biradical itself. In the rigid molecules of the biradicals



(see [77-80]), which contain unsaturated double or triple bonds in the connecting bridges, the indirect mechanism of exchange is observed at even greater distances than in biradicals of types I, II, and XI-XX.

The available experimental data make it possible to estimate the "quenching" ( $\gamma$ ) of the exchange integral  $J$  with delocalization of the unpaired electrons through different atoms or groups of atoms and bonds. An analysis of the data in [18, 61, 70, 79, 80] is given in Table 5. For the biradicals II, XI-XX, the calculated parameter  $\gamma$  is the ratio of the values of the exchange integrals of the individual conformations, and for the biradicals XXVII-XXXI it is the ratio of the values of the average exchange integrals  $\bar{J}$  at the same temperature (in these biradicals, rapid spin exchange is observed). In principle, the last comparison is not a strictly correct procedure, since the exact values of  $J_j$  and  $P_j$  for the corresponding effective conformations are not known, and a detailed study of the temperature dependence of  $\bar{J}$  is necessary. In view of the weak temperature dependence of  $\bar{J}$  in the range 15-65°C for the biradicals XXVII-XXXI [79-80], however, the calculated values of  $\gamma$  are apparently fairly accurate.

For the biradicals II and XI-XX the values obtained for  $\gamma$  characterize the decrease in  $J$  relative to the Si (or O) atom; for the biradicals XXVII-XXXI the values of  $\gamma$  characterize the absolute decrease in the exchange integral when the corresponding group of atoms and bonds is introduced into the chain.

To conclude the present section we shall consider possible reasons why the value of  $J$  depends little on temperature and on the nature of the solvent, noted for the biradical II, which has been most extensively studied [30, 48, 61, 70]. It can be seen from Fig. 12 and Table 4 that an appreciable increase (up to 30-40%) in  $|J|$  is observed in strongly polar solvents (water, chloroform, bromoform, acetic acid). In [70] it was pointed out that the most probable reason for the increase in  $J$  in these solvents is an increase in the "electronic conductivity" of the bridge of  $\sigma$  bonds as a consequence of the formation of weak biradical-solvent complexes with hydrogen bonds at the C=O group of the bridge. The importance of complex formation at the C=O group, and not the N-O group, is indicated by the difference in the effects of the solvent on  $J$  and  $a$  (see Table 4). The same qualitative influence of the nature of the solvent on  $J$  is observed for the biradical XX, which is similar to the biradical II [70].

The slight ( $\leq 10\%$  at 100°) increase in the measured value of  $|J|$  with decrease in temperature may be due, on the one hand, to an increase in the extent of the above complex formation, and on the other hand, to the fact that the intramolecular motion in biradical II, which corresponds to the criterion of slow exchange at temperatures  $\geq 0^\circ\text{C}$ , nevertheless leads to slight averaging of the measured value of  $J$ . Finally, a third reason for the increase in  $|J|$  may be that the observed effective conformation with  $|J| \sim 2a$  in fact consists of several



conformations with similar values of  $J$  and that with decrease in temperature a conformation with slightly greater exchange interaction is stabilized.

### 5. Influence of the Interaction of the Molecule of the Biradical with the Solvent on the Dynamics of Conformational Transitions

As already noted, it is most convenient to use as SP biradicals with rapid exchange and  $|\bar{J}| \sim a$ . In [81] a biradical of this kind was used to demonstrate the sensitivity of the thermodynamics of the intramolecular motion of these biradicals to the nature of the matrix. A study was made of the biradical



with rapid exchange and  $|\bar{J}| \sim a$ , readily soluble in water and organic solvents. The temperature dependence of the measured value of  $\bar{J}$  for this biradical in various solvents is shown in Fig. 19. It can be seen from the figure that all the solvents studied can be divided into three groups according to their influence on the value of  $\bar{J}$ : a) polar solvents - alcohols, b) the nonpolar toluene and dioxane, and c) water. The temperature dependence of  $\bar{J}$  is the same within each group, within the limits of experimental error.

It was shown that the observed temperature dependence of  $\bar{J}$  for the biradical XXXII is described fairly effectively by the dynamic model with two conformations. Since the form of the ESR spectra shows that at low temperatures  $|\bar{J}| \ll a$ , it may be assumed that  $|J_1| \ll a$ , so that

$$\bar{J} = J_2 \tau_2 / (\tau_1 + \tau_2) \quad (44)$$

From Eqs. (33) and (44) it follows that

$$\ln \left| \frac{\bar{J}}{a} \right| = \frac{\Delta S}{R} - \frac{\Delta H}{RT} + \ln \left| \frac{J_2 - \bar{J}}{a} \right| \quad (45)$$

so that, in principle, the unknown values of  $J_2$ ,  $\Delta H$ , and  $\Delta S$  can be determined from the deviation of the temperature dependence of  $\bar{J}$  from the Arrhenius relationship at high temperatures. The expressions giving the experimental temperature dependence of  $\bar{J}$  for ethanol, dioxane, and water were treated on a computer by the method of least squares using Eq. (45). The optimum parameters  $\Delta S$ ,  $\Delta H$ , and  $J_2$ , found by minimization of the dispersion

$$\sigma^2 = \left\{ \sum_i [\bar{J}_{\text{expt}}^2(T_i) - \bar{J}_{\text{theor}}^2(T_i)] \right\} / \sum_i \bar{J}_{\text{expt}}^2(T_i),$$

are given in Table 6. It may be noted that  $\Delta H$  is obtained with the greatest accuracy, since it is determined chiefly by the slope of the curves at low temperatures (see Fig. 19). The value of  $J_2$  is obtained with the lowest (logarithmic) accuracy, since the observed deviations of the temperature dependence of  $\bar{J}$  from the Arrhenius relationship are small.

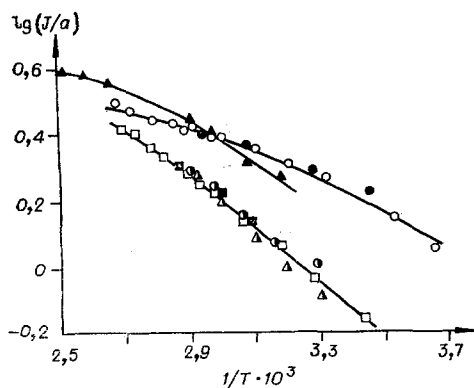


Fig. 19. Temperature dependence of the measured value of the exchange integral for the biradical XXXII in different solvents:  $\Delta$ ) water,  $\circ$ ) dioxane,  $\bullet$ ) toluene,  $\circ$ ) methanol,  $\square$ ) ethanol,  $\Delta$ ) n-butyl alcohol,  $\blacksquare$ ) isobutyl alcohol.

TABLE 6. Optimum Values of  $\Delta H$ ,  $\Delta S$ , and  $\log|J_2/a|$  for the Biradical XXXII in Different Solvents

Solvent <sup>†</sup>	$\Delta H \pm 0.2$ , kcal/ mole	$\Delta S \pm 2$ , eu	$\log J_2/a  \pm$ $\pm 0.3$
Dioxane	3.8	12.1	0.62
Ethanol	4.3	10.0	0.94
Water	5.6	16.7	0.70

<sup>†</sup>Within the limits of accuracy of the experiment, the set of optimum parameters found for dioxane also applies to toluene, and that for ethanol also applies to methanol and butanol.

It follows from the data in Table 6 that the values of  $J_2$  are the same in all the solvents, within the limits of accuracy of the measurements. The apparent reason is that in the short biradical XXXII, as in the biradicals I, II, and XI-XX, spin exchange takes place along a chain of  $\sigma$  bonds, and not by collisions of the N-O fragments. The indirect mechanism of exchange in the biradical XXXII is confirmed by the fact that  $J_2 \sim 5 \cdot 10^8$  rad/sec is appreciably smaller than the value  $10^{10}$ - $10^{11}$  rad/sec, characteristic of exchange involving collisions (see above).

The nature of the solvent has a considerable influence on the values of  $\Delta H$  and  $\Delta S$ . The difference in the enthalpies  $\Delta H$  measured in nonpolar solvents is close to the value characteristic of rotational stereoisomers. At the same time, in polar solvents (particularly alcohols and water), the molecules of which can form hydrogen bonds with the molecule of the biradical XXXII, the difference in the enthalpies of the conformations "1" and "2" is much greater. A noteworthy feature is the large difference in the entropies of the conformations in all the solvents. This probably indicates that the conformation "2" is much less extensively solvated than the conformation "1."

## 6. Summary

To conclude the present section, we can summarize briefly the conclusions regarding the information which it is possible in principle to obtain by studying the isotropic ESR spectra of liquid solutions of nitroxide biradicals.

1. It is possible to observe transitions between conformations with different values of the exchange integral, and it is possible to determine the minimum number of conformations between which transitions are observed.
2. For the observable conformational transitions it is possible to reach unambiguous conclusions as to whether they correspond to the conditions of rapid ( $\nu > 5 \cdot 10^7$  Hz) or slow ( $\nu < 5 \cdot 10^7$  Hz) exchange.
3. In the case where slow conformational transitions are observed, it is possible to determine the relative statistical weights of the effective conformations, and in a number of cases this makes it possible to establish the differences in the enthalpies and entropies of these conformations.
4. In the case where rapid intramolecular motion is observed from the alternated broadening of the ESR lines it is possible to determine the characteristic frequency of this motion and its corresponding activation energy. Unfortunately, the values determined cannot be related unambiguously to the time parameters of the conformational transitions.
5. In the case where the observed rapid motion corresponds to the averaged value of the exchange integral  $|\bar{J}| \sim a$ , it is usually possible to estimate the difference in the enthalpies and entropies of the conformations between which the transitions take place, and also to estimate the magnitude of the exchange integral in the corresponding separate effective conformations.
6. If the ESR spectra make it possible to carry out accurate measurements of the value of the exchange integral, it is possible in a number of cases to reach a sufficiently unambiguous conclusion regarding the way in which the exchange interactions take place, and in some cases to relate the observed values of the exchange integral to a definite three-dimensional biradical structure.

7. Comparison of the dynamic and thermodynamic parameters of intramolecular motion in different solvents makes it possible to reach conclusions regarding the extent of the solvation of the molecule of the biradical and the part played by its influence on the different types of intramolecular motion.

### III. THE STUDY OF BIRADICALS IN DILUTE VITREOUS SOLUTIONS

When a solution is frozen, the ESR spectra of the biradicals reveal not only the isotropic exchange interaction but also the magnetic dipole-dipole interaction of the radical fragments. Since the magnitude of this interaction is closely related to the distance between the paramagnetic centers of the biradicals (it is understood that the solutions of the biradicals are sufficiently dilute to exclude the possibility of dipole-dipole interaction between different molecules), the correct interpretation of the ESR spectra of biradicals in frozen solutions makes it possible to obtain extremely important information on the structure of the biradical and on the influence of the solvent matrix on this structure.

The forms of the ESR spectra of vitreous solutions of nitroxide biradicals are extremely varied (see Figs. 22, 26-28). Nevertheless, the determination of the magnitude of the dipole-dipole interaction from these spectra now presents no particular difficulty, since several independent methods of measurement are known. The determination of the relative spatial orientation of the paramagnetic fragments and the magnitude of the intramolecular exchange interactions in vitreous solutions is much more complicated; even these problems, however, are now being successfully resolved.

#### 1. Influence of Dipole - Dipole Interaction on the ESR Spectrum of a Radical Pair in a Vitreous Solution

The simplest model which makes it possible to follow the influence of the magnetic dipole-dipole interaction in biradicals on the ESR spectrum is the model of a radical pair† with paramagnetic centers exhibiting low anisotropy (compared with the magnitude of the dipole-dipole interaction) of the g factor and the magnitude of the HFI.

The Hamiltonian of the dipole-dipole‡ interaction of the pair can be written as follows [88-90]:

$$\hat{V}_{dd} = g_e^2 \beta_e^2 \left\{ \frac{\mathbf{S}^{(1)} \mathbf{S}^{(2)}}{r_{12}^3} - \frac{3(\mathbf{r}_{12} \mathbf{S}^{(1)}) (\mathbf{r}_{12} \mathbf{S}^{(2)})}{r_{12}^5} \right\}, \quad (46)$$

where  $\mathbf{r}_{12}$  is the vector connecting the two electrons "1" and "2", and the operators  $\mathbf{S}^{(1)}$  and  $\mathbf{S}^{(2)}$  relate to the first and second electrons.

In the case being considered, as a consequence of strong electron-electron diffraction, the "correct" spin wavefunctions are described by the total spin  $\mathbf{S} = \mathbf{S}^{(1)} + \mathbf{S}^{(2)}$ . For radicals with electron spin  $\mathbf{S}^k = 1/2$  there exist one singlet wavefunction with  $\mathbf{S}_{\text{total}} = 0$  ( $\psi_S = (1/\sqrt{2})(\alpha\beta - \beta\alpha)$ ) and three triplet functions with  $\mathbf{S}_{\text{total}} = 1$ :

$$\psi_T(-1) = \beta\beta, \quad \psi_T(0) = (1/\sqrt{2})(\alpha\beta + \beta\alpha) \text{ and } \psi_T(1) = \alpha\alpha. \quad (47)$$

In ESR, transitions are observed between the triplet wavefunctions: two allowed transitions with  $\Delta M_S = 1$  (the transitions  $\psi_T(-1) \leftrightarrow \psi_T(0)$  and  $\psi_T(0) \leftrightarrow \psi_T(1)$ ) and one "forbidden" transition with  $\Delta M_S = 2$  (the transition  $\psi_T(-1) \leftrightarrow \psi_T(1)$ ).

By selecting the "dipole" system of coordinates in such a way that the Z axis is directed along the vector  $\mathbf{r}_{12}$ , it is possible to obtain for the ns electrons from the Hamiltonian (46) a spin Hamiltonian of simple form [88-90]

$$\mathcal{H}_{dd} = D \left( S_z^2 - (1/3) S(S+1) \right), \quad (48)$$

where

† The polycrystalline ESR spectra of triradicals are discussed in [82-86].

‡ Here and subsequently we shall not consider the pseudodipole part of the electron-electron interaction [15, 44, 87]  $D_{\text{pseudo}} = (-1/8)J \left\{ (1/4)(g_{\parallel} - 2)^2 (g_{\perp} - 2)^2 \right\}$ , which for the values of the anisotropy of the g factors ( $\Delta g < 0.01$ ) and values of the exchange integrals ( $|J| \leq 10^{10}$  Hz) typical of nitroxide biradicals is appreciably smaller than the magnitude of the true dipole-dipole interaction.

$$D = \frac{3}{2} g_e^2 \beta_e^2 / r_{12}^3 \quad (49)$$

(D is expressed in frequency units.)

In the general case, when the unpaired electrons cannot be represented in the form of point dipoles, the spin Hamiltonian of the dipole-dipole interaction can be obtained using the one-electron molecular orbitals of the first and second radical center (*a* and *b* respectively) [88, 89, 91]. The complete triplet wavefunction  $\Psi$  is equal to the product of the antisymmetric spatial wavefunction

$$\varphi = (1/\sqrt{2})[a(1)b(2) - b(1)a(2)]$$

(the figures in parentheses relate to the electrons) and the spin triplet function (47)

$$\Psi = \varphi \cdot \psi_T(M_S),$$

where  $M_S = -1, 0, 1$ . In the case of real functions *a* and *b* the spin Hamiltonian  $\mathcal{H}_{dd}$ , obtained from the Hamiltonian (46), has the form

$$\mathcal{H}_{dd} = \langle a(1)b(2) | \hat{V}_{dd} | a(1)b(2) \rangle - \langle b(1)a(2) | \hat{V}_{dd} | a(1)b(2) \rangle. \quad (50)$$

The spin Hamiltonian (50) represents a matrix containing all possible combinations of paired products  $S_i S_j$ , where *i, j* = *x, y, z*. By diagonalizing this matrix it is possible to find the principal "dipole" system of coordinates *X, Y, Z* in which the spin Hamiltonian takes the simple form

$$\mathcal{H}_{dd} = D \left( S_Z^2 - \frac{1}{3} S(S+1) \right) + E (S_X^2 - S_Y^2). \quad (51)$$

In the principal system of coordinates

$$D = (3/4) g_e^2 \beta_e^2 \left\langle \varphi \left| \frac{1}{r_{12}^3} - \frac{3z_{12}^2}{r_{12}^5} \right| \varphi \right\rangle \quad (52)$$

and

$$E = (3/4) g_e^2 \beta_e^2 \left\langle \varphi \left| \frac{y_{12}^2 - x_{12}^2}{r_{12}^5} \right| \varphi \right\rangle, \quad (53)$$

where  $x_{12}, y_{12},$  and  $z_{12}$  are the components of the vector  $\mathbf{r}_{12}$ . In the case where the two radical centers are separated from one another by a distance which is greater than the dimensions of the range of delocalization of the unpaired electrons, the axis *Z* is directed along the straight line joining approximately the centers of gravity of the clouds of the unpaired electrons. It can be seen from Eq. (53) that for radical pairs exhibiting a sufficiently high symmetry with respect to the *Z* axis,  $E = 0$ .

In principle, the values of *D* and *E* can be calculated with a high accuracy by examining only the two-center Coulomb integrals. Here it is possible to use data on the distribution of the spin density ( $\rho_i$ ) of the unpaired electrons and model descriptions of the atomic orbitals [10, 91].

In fact, in the case where the unpaired electrons are localized on different molecular groups, the second term in Eq. (50) can be neglected, so that  $\mathcal{H}_{dd}$  can be calculated as the classical dipole-dipole interaction between two dipoles distributed in space with a probability density equal to the probability density of finding the unpaired electron at a given point. For the simplified calculation of  $\mathcal{H}_{dd}$  in the case where the molecular orbitals are represented in the form of LCAO, it was suggested in [28] that simple models be used to replace the real three-dimensional electron density distribution of the atomic orbitals (AO). In particular, it is suggested that the "dumbbell" np-AO (the unpaired electrons in organic radicals are generally distributed in 2p-AO [4]) be replaced by two point half-charges  $q_{il} = \rho_i/2$ , situated at a certain distance  $h_l$  on either side of the atom "i" along the direction of the np-AO ( $l = 1, 2$ ).

From Eqs. (46) and (50) in this case we obtain the simple expression

$$\mathcal{H}_{dd} = g_e^2 \beta_e^2 \sum_{\substack{i,j \\ l,p}} q_{il}^{(1)} q_{jp}^{(2)} \left\{ \frac{\mathbf{S}^{(1)} \mathbf{S}^{(2)}}{r_{ij}^3} - \frac{3 (r_{ij} \mathbf{S}^{(1)}) (r_{ij} \mathbf{S}^{(2)})}{r_{ij}^5} \right\}, \quad (54)$$

where the summation is carried out over all the charges modeling the AO,  $\mathbf{r}_{ij}$  is the vector connecting the charges  $q_{il} q_{jp}$  ( $l, p = 1, 2$ ), and the superscript relates to the corresponding radical center. The spin Hamiltonian (54) is brought to the form (51) by the method described above.

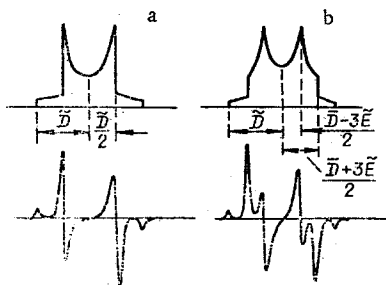


Fig. 20. Absorption spectrum (above) and its first derivative (below) for a radical pair with an isotropic  $g$  factor without HFI: a)  $E = 0$ , b)  $E \neq 0$ .

The complete spin Hamiltonian of the radical pair (the HFI is here regarded as a perturbation) is written in the form [29, 88, 93]

$$\mathcal{H} = \beta_e H_0 \hat{g} \mathbf{S} + D(S_z^2 - (1/3)S(S+1)) + E(S_x^2 - S_y^2). \quad (55)$$

By solving the cubic equation corresponding to the secular determinant of the spin Hamiltonian (55), we find that the transitions  $\psi_T(-1) \leftrightarrow \psi_T(0)$  and  $\psi_T(0) \leftrightarrow \psi_T(1)$  correspond to two magnetic field intensities  $H_1$  and  $H_2$  respectively. In the case where the ESR is observed at a constant frequency  $\nu_0$  of a superhigh frequency field, the position of the lines of the spectra in the zeroth approximation is given by the expression [89]

$$H_{1,2} = H \pm \{(\tilde{D} - 3\tilde{E})^2/2 + (\tilde{D} + 3\tilde{E})m^2/2 - \tilde{D}n^2\}^{1/2}, \quad (56)$$

where  $H = h\nu_0/g\beta_e$ ,  $\tilde{D} \equiv D/g_e\beta_e$ ,  $\tilde{E} \equiv E/g_e\beta_e$ ,  $g = [g_{XX}^2 l^2 + g_{YY}^2 m^2 + g_{ZZ}^2 n^2]^{1/2}$  and  $l$ ,  $m$ , and  $n$  are the direction cosines of the dipole system of coordinates relative to the external magnetic field. For ESR in the X range ( $\nu_0 \sim 9.5$  GHz) the visible dipole-dipole splitting  $d = |H_1 - H_2|$  is described sufficiently effectively by the zeroth approximation even for large values of the dipole-dipole interaction [29, 94]

$$d(\theta, \varphi) = |\tilde{D}(3\cos^2\theta - 1) + \tilde{E} \cdot 3\sin^2\theta \cos 2\varphi|. \quad (57)$$

Here,  $\theta$  and  $\varphi$  are the spherical coordinates of the dipole system of coordinates relative to the direction of the constant magnetic field  $H_0$ .

Thus in the case of a single crystal the Z axis of the dipole system determines the maximum splitting  $|2\tilde{D}|$ , the X axis the splitting  $|-\tilde{D} + 3\tilde{E}|$  and the Y axis  $|\tilde{D} + 3\tilde{E}|$ .

The polycrystalline ESR spectra of radical pairs, taking account of the random orientation of the radical pairs in polycrystalline specimens, were calculated in [88]. The absorption spectra corresponding to the cases  $E=0$  and  $E \neq 0$  are given in Fig. 20. It can be seen that in the case where  $E=0$  the ESR spectrum of the pair should show two "parallel" components of the spectrum with a splitting between them  $|2\tilde{D}|$ , corresponding to the orientation of the pairs  $Z \parallel H_0$ , and two "perpendicular" components with a splitting between them  $|\tilde{D}|$ , corresponding to the orientation  $Z \perp H_0$  (see Fig. 20a). In the case where  $E \neq 0$ , each "perpendicular" component is split into two (Fig. 20b).

The observed magnitude of the components of the  $g$  tensor ( $\bar{g}$ ) is calculated from the average field intensity  $\bar{H} = (H_1 + H_2)/2$  and the relationship  $h\nu_0 = \bar{g}\beta_e \bar{H}$ . In the case where the dipole-dipole interactions are sufficiently large, the true values of  $g_{XX}$ ,  $g_{YY}$ , and  $g_{ZZ}$  (it should be remembered that these elements of the  $g$  tensor here correspond to the dipole system of coordinates and may not be the characteristic values of the  $g$  tensor) can be found from the experimentally determined values of  $\bar{g}_{XX}$ ,  $\bar{g}_{YY}$ ,  $\bar{g}_{ZZ}$ ,  $\tilde{D}$ , and  $\tilde{E}$  by means of the simple expressions [29, 95]

$$\begin{aligned} g_{XX} &= \bar{g}_{XX} \{1 - (\tilde{D} + \tilde{E})^2/8\bar{H}^2\}, \\ g_{YY} &= \bar{g}_{YY} \{1 - (\tilde{D} - \tilde{E})^2/8\bar{H}^2\}, \\ g_{ZZ} &= \bar{g}_{ZZ} \{1 - \tilde{E}^2/2\bar{H}^2\}. \end{aligned} \quad (58)$$

The problems associated with the procedure for determining the parameter  $D$  in the case of uniform and nonuniform broadening of the ESR spectra of radical pairs, and also in the case of saturation of the signal  $\Delta M_S = 1$ , are examined in [91, 96-98].

If it is assumed for simplicity that the two radical fragments are oriented in symmetrical fashion relative to the axes X, Y, and Z, and if the hyperfine structure from the  $^{14}\text{N}$  nucleus is regarded as a perturbation [29], it is found that the X, Y, and Z components of the ESR spectra are split into three lines of equal intensity (the case  $|J| \ll |A_{ii}|$ , where  $A_{ii}$  represents the components of the HFI tensor) or five lines with intensity ratio 1:2:3:2:1 (case  $|J| \gg |A_{ii}|$ ) (the case  $D \sim |J| \sim |A_{ii}|$  is examined in a separate section). Here, the mag-

nitude of the splitting is equal to  $A_{XX}$ ,  $A_{YY}$ , and  $A_{ZZ}$  or  $A_{XX}/2$ ,  $A_{YY}/2$ , and  $A_{ZZ}/2$  respectively (the components of the A tensor, like the components of the g tensor, are here given not in the principal axes of coordinates of these tensors, but in the axes of the dipole system of coordinates).

The existence of the dipole-dipole interaction makes the "forbidden" transition with  $\Delta M_S=2$  allowed [90, 99, 100]. As a consequence of this, in the "half" field [101]

$$H_{00} = [\omega_0^2 - (4/3) D^2]^{1/2} / 2g\beta_e \quad (59)$$

a weak signal corresponding to  $g \approx 4$  is observed. The form of this signal corresponds to the signal with  $\Delta M_S = 1$  in the absence of dipole-dipole interaction [28, 90, 102].

The ratio of the integral intensities  $I_1$  and  $I_2$  of the signals with  $\Delta M_S = 1$  and  $\Delta M_S = 2$  depends on the magnitude of D [99, 100]; for a polycrystalline specimen this ratio is given by [44, 100, 103]

$$\alpha = \frac{I_2}{I_1} = \frac{2}{15} \left( \frac{D}{g\beta_e H_{00}} \right)^2 \quad (60)$$

For  $|J| \sim kT$ , the two integral intensities  $I_1$  and  $I_2$  show the same temperature dependence (see footnote to p. 108), so that  $\alpha$  should be independent of temperature.

## 2. Electronic Structure and Anisotropy of the $\hat{g}$ and $\hat{A}$ Tensors of the N - O Fragments of Nitroxide Radicals and the Relationship between the Parameter D and the Distance between the N - O Fragments

An important feature facilitating the interpretation of the polycrystalline ESR spectra of nitroxide biradicals is that in the study of the ESR spectra of single crystals [4] and x-ray diffraction data [4, 104] it has been established that the N-O fragments in nitroxide biradicals have the same electronic structure as the N-O fragments of the corresponding monoradicals, and hence analogous parameters of the  $\hat{g}$  and  $\hat{A}$  tensors. It has been established that the N-O bond can with a sufficient degree of accuracy be regarded as lying in the plane C-N-C [4, 104], and that the unpaired electron is practically completely localized in the 2p orbitals of the N and O atoms (the spin densities on the atoms are  $\rho_N = 0.3 \pm 0.1$  and  $\rho_O = 0.7 \pm 0.1$  [4] respectively).

As a consequence of this, the principal axes of the  $\hat{g}$  and  $\hat{A}$  tensors practically coincide and can be related to the structure of the N-O fragment as shown in Fig. 21a. Typical values of the parameters of these tensors (for the case of the di-t-butyl radical [4, 105]) are as follows:

$$g_{xx} = 2.0061, \quad g_{yy} = 2.0089, \quad g_{zz} = 2.0027, \\ A_{xx} = 7 \text{ G}, \quad A_{yy} = 5 \text{ G}, \quad A_{zz} = 32 \text{ G}.$$

(the values of the analogous parameters for other nitroxide radicals are indicated in [30, 106-111]).

As shown above (Eqs. (52)-(54)), the values of D and E depend on the distribution of the electron density of the unpaired electrons between the paramagnetic centers. As a consequence of this, the distance r determined from Eq. (49) is a quantity characterizing the paramagnetic centers as a whole. The relationship between the magnitude of r and the distances between the separate parts of the paramagnetic N-O fragments was discussed in [28, 92, 102, 112].

It was shown that for  $r \geq 4 \text{ \AA}$  the magnitude of r determined from Eq. (49) corresponds with a high accuracy to the distance between the centers of the N-O fragments.

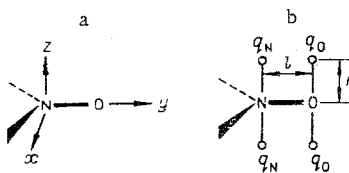


Fig. 21. Principal axes of the  $\hat{g}$  and  $\hat{A}$  tensors of the N-O fragment in nitroxide radicals (a) and the modeling of the electron density distribution in the N-O fragment by means of four point charges (b).

For a more detailed interpretation of the magnitude of  $r$ , a description of the N-O fragment based on the Heitler - London approximation was proposed [28, 92]. The wavefunction  $\varphi$  in this approximation is given by

$$\varphi = \frac{1}{\sqrt{2}} [\pi_A^*(1) \pi_B^*(2) - \pi_B^*(1) \pi_A^*(2)],$$

where  $\pi_A^*$  and  $\pi_B^*$  represent the antibonding  $\pi$ -molecular orbitals of the two unpaired electrons on each N-O group. For the group  $k$  ( $k=A$  or  $B$ ) in the simplest approximation of LCAO, the antibonding orbital  $\pi_k^*$  is described by the following expression:

$$\pi_k^* = \sqrt{\rho_{N_k}} (2p_{yN_k}) - \sqrt{\rho_{O_k}} (2p_{yO_k}),$$

where  $\rho_{N_k}$  and  $\rho_{O_k}$  are the electron populations of the  $2p_y$  orbitals of the  $N_k$  and  $O_k$  atoms respectively. Figure 21b gives a simplified model (see p. 132) of the distribution of electric charges in the N-O fragment. Agreement between the calculated and experimentally measured values of  $D$  was observed [28, 92] for  $q_N = 0.135-0.15$ ;  $q_O = 0.365-0.35$ ,  $l = 1.3 \text{ \AA}$ ;  $h = 0.6-1 \text{ \AA}$ .

### 3. Interpretation of the Experimental ESR Spectra of Vitreous Solutions of Nitroxide Biradicals with Large $D$

The theory of the polycrystalline ESR spectra of radical pairs given above was used in [28, 29, 92, 94, 102, 113, 114] to find the three-dimensional structure of the conformations of nitroxide biradicals stabilized in glasses.

As a typical example we shall examine the interpretation of the ESR spectra of a dilute solution of the biradical XXIII in *n*-butyl alcohol at  $-115^\circ\text{C}$  [102] (Fig. 22). In the spectrum of the allowed transition  $\Delta M_S = 1$  we can readily distinguish groups of lines relating to the situation where the external field  $H_0$  is parallel to the axes  $X$ ,  $Y$ , or  $Z$  of the tensor of the dipole-dipole interaction. We can examine these groups of lines.

a. The two outer lines of the spectrum with a splitting between them  $242 \pm 2 \text{ G}$  correspond to the situation where  $H_0 \parallel Z$ . Thus  $\tilde{D} = 121 \pm 1 \text{ G}$ . The center of gravity of these two lines corresponds to  $g_{ZZ} = 2.0063 \pm 0.0002$ , which is close to the value of  $g_{XX}$  for the corresponding monoradicals [101, 105]. This apparently indicates that  $Z$  is directed along the  $x$  axis of the  $\hat{g}$  tensor. Since from Eq. (49)  $r = 6.12 \text{ \AA}$  and with allowance for the above remarks regarding the relationship between  $r$  and the distance between the N-O fragments, we can put forward for the biradical XXIII the three-dimensional structure indicated in Fig. 22.

b. The remaining lines of the spectrum correspond to the situation  $H_0 \parallel X$  and  $H_0 \parallel Y$ . The remaining lines in the spectrum are: A) two intense lines with a width of approximately 8 G, separated by 134 G, and B) five lines of lower intensity on either side of the spectrum (with a linewidth of approximately 5 G).

This indicates that the triplet does not have axial symmetry ( $E \neq 0$ ). From Eqs. (57) we conclude that  $(\tilde{D} + 3\tilde{E}) = 134 \pm 2 \text{ G}$ ,  $(\tilde{D} - 3\tilde{E}) = 112 \pm 2 \text{ G}$ , that is,  $\tilde{E} = 3.5 \pm 1 \text{ G}$  and  $\tilde{D} = 123 \pm 2 \text{ G}$ , is followed from the lines corresponding to  $Z \parallel H_0$ .†

† According to the data in [29], the interstitial single crystal for this biradical shows two conformations, one with  $2\tilde{D} = 246 \pm 3 \text{ G}$  and  $E = 2.2 \pm 0.5 \text{ G}$ , and the other with  $2\tilde{D} = 393 \pm 5 \text{ G}$  and  $E = 2.0 \pm 0.5 \text{ G}$ , which corresponds to  $r = 6.09$  and  $5.20 \text{ \AA}$  respectively.

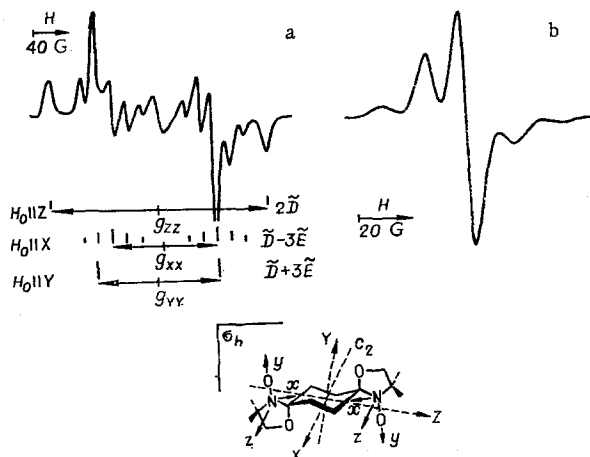


Fig. 22. Allowed (a) and "forbidden" (b) ESR spectra of a *n*-butyl alcohol solution of the biradical XXIII, vitrified at  $-115^\circ\text{C}$ , and its conformational structure corresponding to the given spectra.

The corresponding values of  $g$  for the groups of lines A and B are  $g_A = 2.0085 \pm 0.0002$  and  $g_B = 2.0026 \pm 0.0002$ . The average value  $g_{aver} = 1/3(g_{ZZ} + g_A + g_B) = 2.0058 \pm 0.0002$  agrees with the value  $g_0 = 2.0061 \pm 0.0002$ , obtained in the liquid solution. Comparison of  $g_A$  and  $g_B$  with the known values of  $g_{yy} = 2.0089 \pm 0.0001$  and  $g_{zz} = 2.0021 \pm 0.0001$  for the corresponding monoradical indicates that the group of lines A should be assigned to the situation  $H_0 \parallel Y$ , and the group B to the situation  $H_0 \parallel X$  (see Fig. 22).

In the multiplets B, which correspond to  $H_0 \parallel X$ , five lines separated by 16 G are observed; the intensities of these lines correspond approximately to the ratio 1:2:3:2:1. Thus this biradical corresponds to the case of strong exchange ( $|J| \gg |A_{ii}|$ ) and  $A_{XX} = 32$  G. Since in liquid solution for this biradical  $a = 14.5$  G, it follows that  $A_{ZZ}$  and  $A_{YY}$  should be equal to approximately 6 G ( $A_{ZZ} \approx A_{YY} = (3a - A_{XX})/2$ ), that is all the lines corresponding to  $H_0 \parallel Y$  and  $H_0 \parallel Z$  should be split into five components with a splitting of 3 G. The observed line-width  $\sim 8-9$  G agrees with this conclusion.

An interesting feature is that the simplicity of the analysis of the hyperfine structure of this spectrum became possible as a result of the fact that the biradical XXIII is stabilized in a symmetrical conformation with axes X, Y, and Z directed approximately along the principal axes of the  $\hat{g}$  tensors.

The forbidden transitions  $\Delta M_S = 2$  for the biradical XXIII are observed in a field of 1670 G and have a five-line HFS (see Fig. 22), which corresponds, as it should, to the case of strong exchange. The fact, observed in [102], that the intensity of the spectrum of the forbidden transition is independent of temperature in the range  $123^\circ\text{K} < T < 223^\circ\text{K}$  indicates that for the conformation of biradical XXIII with  $r = 6.12 \text{ \AA}$  the magnitude of the exchange integral  $|J| < 20^\circ\text{K}$ .

The intensity of the forbidden signal  $\Delta M_S = 2$  was found to be dependent on temperature [28] for the biradical XXIV with  $r = 4.8 \text{ \AA}$ . Here it was found that  $J = -170 \pm 15^\circ\text{K}$ , that is, the ground state of the biradical XXIV is a triplet.

#### 4. Interpretation of the Polycrystalline ESR Spectra of Nitroxide Biradicals with $|J|$ and/or $|D| \sim |A_{ii}|$

All the recommendations given above on the procedure for analyzing polycrystalline ESR spectra of nitroxide biradicals become inapplicable when the order of magnitude of  $|J|$  and/or  $|D|$  is the same as that of  $|A_{ii}|$ , which is always the case at  $r \geq 9 \text{ \AA}$ . In this, firstly, the wavefunctions of the biradical cannot be divided into "purely" triplet and "purely" singlet functions (see Eq. (5)) and, secondly, it is necessary to take account of the mutual orientation of the radical fragments relative to one another and relative to the dipole system of coordinates. This orientation is given by means of five independent (and unknown beforehand) Eulerian angles  $\alpha_1, \beta_1, \alpha_2, \beta_2$ , and  $\gamma_2 - \gamma_1$  (Fig. 23).

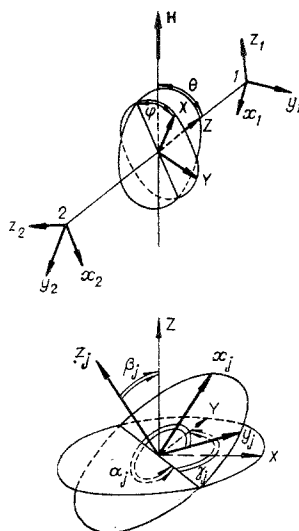


Fig. 23. Parameters of the spatial orientation of the radical fragments in the molecule of the biradical.



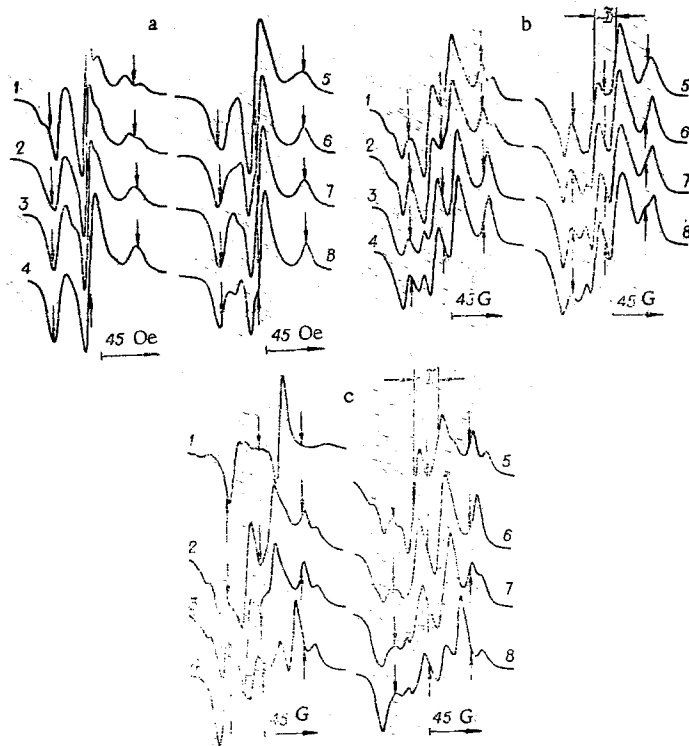


Fig. 24. Form of the polycrystalline ESR spectra of nitroxide biradicals for different relative orientations of the radical fragments for  $\tilde{D}=5$  G (a),  $\tilde{D}=17$  G (b), and  $\tilde{D}=20$  G (c). For all the spectra,  $J=0$ . Here and subsequently, the arrows indicate the positions of the components of the ESR spectrum of the monoradical (see Fig. 28), calculated for the same values of the parameters of the  $\hat{g}$  and  $\hat{A}$  tensors.

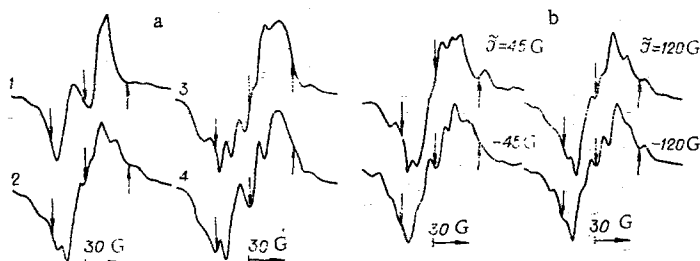


Fig. 25. Dependence of the form of the polycrystalline ESR spectrum of a nitroxide biradical on the relative orientation of the radical fragments with exchange interaction  $\tilde{J}=90$  G (a) and on the magnitude and sign of the exchange integral for the same relative orientation of the radical fragments (b). For all the spectra,  $\tilde{D}=20$  G.

Since the parameter  $E$  is small compared with  $D$ , the complete spin Hamiltonian of the biradical can be written in the form [30, 115]

$$\begin{aligned} \hat{\mathcal{H}} = & \beta_e H_0 \hat{g}^{(1)} S^{(1)} + \beta_e H_0 \hat{g}^{(2)} S^{(2)} + S^{(1)} \hat{A}^{(1)} I^{(1)} + S^{(2)} \hat{A}^{(2)} I^{(2)} + \\ & + JS^{(1)} S^{(2)} + D_0 \left\{ \frac{3}{2} S_z^{(1)} S_z^{(2)} - \frac{1}{2} S^{(1)} S^{(2)} \right\}. \end{aligned} \quad (61)$$

In Eq. (61) the quantization of the electron spin is carried out along the direction of the field  $H_0$ , and not along an axis of the dipole system of coordinates, so that  $D_\theta = D(1 - 3 \cos^2 \theta)$ .

The calculation of the corresponding energy levels and the probabilities of the transitions for arbitrary values of  $\hat{g}$ ,  $\hat{A}$ ,  $D$ ,  $J$  and  $\alpha$ ,  $\beta$ ,  $\gamma$  is given in [115]. The numerical calculation of the polycrystalline spectra

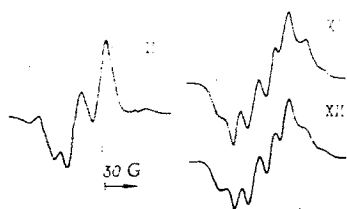


Fig. 26

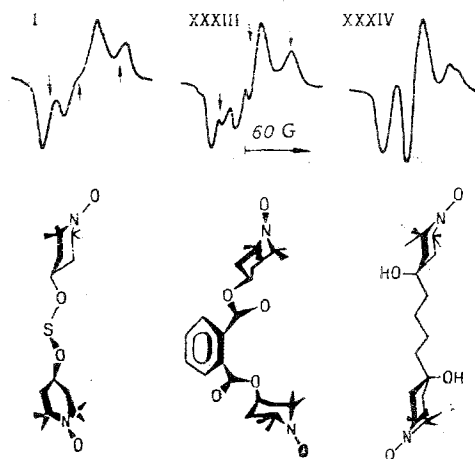


Fig. 27

Fig. 26. ESR spectra of toluene solutions of biradicals II, XI, and XII, vitrified at 77°K.

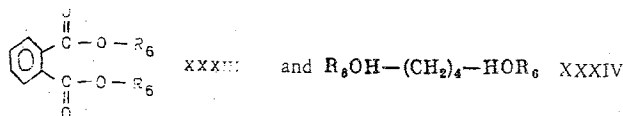
Fig. 27. ESR spectra of toluene solutions of the biradicals I, XXXIII, and XXXIV vitrified at 77°K, and the suggested conformational structures corresponding to these spectra.

TABLE 7. Parameters of the Relative Orientation of the Radical Fragments of the Biradicals I, XXXIII, and XXXIV [115]

Biradical	$r, \text{Å}$	$\beta_1/\pi$	$\beta_2/\pi$	$(\gamma_2 - \gamma_1)/\pi$
I	$11,8 \pm 0,2$	$0,3 \pm 0,1$	$0,7 \pm 0,1$	$0,5 \pm 0,25$
XXXIII	$11,8 \pm 0,2$	$0,35 \pm 0,05$	$0,65 \pm 0,05$	$0,5 \pm 0,1$
XXXIV	$13,8 \pm 0,3$	0,54	0,54	1

under these conditions indicates that the form of the spectrum depends to a very marked extent on the relative orientation of the radical fragments, and also on the magnitude and sign of  $J$  (Figs. 24 and 25). It was shown that from the presence of the characteristic lines in the ESR spectrum of the biradical it is possible to reach qualitative conclusions regarding the magnitude of  $|J|$ . Thus for  $\tilde{D} \sim 15\text{--}20 \text{ G}$  ( $r \sim 10\text{--}12 \text{ Å}$ ) in the case where  $|\tilde{J}| \geq 40 \text{ Hz}$  (here and subsequently  $\tilde{J} = J/g_e\beta_e$ ) either a quartet of lines in the center of the spectrum (Fig. 25a) or characteristic satellites (Fig. 25a, curve 1) are observed. Comparison of the experimental ESR spectra of the biradicals II, XI, and XII (Fig. 26) with the theoretical spectra in Fig. 25 indicates that even in the vitrified solutions there is appreciable exchange interaction ( $J \geq 40 \text{ G}$ ) between the radical fragments of these biradicals (this conclusion provides direct confirmation of the indirect mechanism of exchange in II, XI, and XII).

When the condition  $|\tilde{J}| \ll 40 \text{ G}$  is fulfilled, the biradical ESR spectra make it possible to determine the values of  $r$ , and also the angles  $\beta$  and  $\gamma$ . Table 7 gives these parameters for the three biradicals studied in [115], biradical I,



vitrified in toluene at 77°K. The corresponding experimental polycrystalline spectra of these biradicals are given in Fig. 27. Figure 27 also gives the three-dimensional structure of the conformations of these biradicals, defined by means of the parameters  $r$ ,  $\beta$ , and  $\gamma$ .

From the data in [115] it follows that the biradical XXXIV has practically the same structure in the vitrified solution and in the single crystal [116]. This possibility of obtaining experimental data on the structures of different nitroxide biradicals directly in frozen solutions apparently makes it possible to observe and interpret even the small changes taking place in the biradicals when the system being studied is subjected to various influences.

TABLE 8. Comparison of the Distances  $r$  between the N-O Fragments, Found by Different Methods from the ESR Spectra of Solutions Vitrified at 77°K (the solvent is indicated in parentheses: t - toluene, e - ethanol, m - methanol).

Biradical	$r, \text{Å}^a$	$r_M, \text{Å}^b$	$r_S, \text{Å}^c$	$r_D, \text{Å}^d$	$r_{\text{computer}}, \text{Å}^e$	$r_d, \text{Å}^f$
I(t)	—	11,1	10,8	11,6	11,8	—
XI(t)	—	10,8	—	12,0	—	—
XII(t)	—	10,7	10,3	11,9	—	—
XIII(t)	—	10,6	—	10,6	—	—
XIV(t)	—	10,3	10,2	10,9	—	—
XV(t)	—	10,7	—	10,7	—	—
XVI(t)	—	10,0	—	11,6	—	—
II(t)	11,3	11,2	11,7	—	—	—
XLIV(t)	—	9,1	9,3	—	—	—
XXXII(t)	—	10,8	11,5	—	—	—
XXXIII(t)	—	11,5	11,2	12,7	11,8	—
XLV* (t)	18,5	—	—	—	17,8	19,5
XLVI†(e)	13,1	12,9	—	—	—	—
XXXIV(e)	13,2	13,6	—	—	13,8	—
VII(m)	—	13,9	—	—	—	13,0
VIII(m)	—	10,5	—	—	—	10,7
IX(m)	—	11,5	—	—	—	11,3
XXXV(t)	—	15,5	—	—	15,7	14,9

a)  $r$  was calculated from x-ray diffraction data [104, 116] for single crystals as the distance between the centers of the N-O fragments; b) from the data in [61, 121, 123]; c) from the data in [103]; d) from the data in [61]; e)  $r_{\text{computer}}$  was obtained by comparing the experimental spectra with the spectra calculated on a computer [115, 122]; f) from the data in [121, 123].

\* Radical XLV  $R_6\text{OOC}(\text{CH}_2)_6\text{COOR}_6$ .

† Radical XLVI  $R_6(\text{CH}_2)_4R_6$ .

In practice, the magnitude of  $r_S$  is usually determined from the dependence of  $\alpha$  on the concentration  $C$  of the biradical [103]:

$$\alpha = 38/r_S^6 + 160C/R^3. \quad (63)$$

Here,  $R(\text{Å})$  has the significance of the distance of greatest approach of the paramagnetic centers of the two biradicals. Measurement of  $\alpha$  makes it possible to calculate two important characteristics of the molecules  $r_S$  and  $R$ , but extremely high concentrations of the biradicals are required ( $C \geq 10^{19}$  spin/cm<sup>3</sup>). In [103] this method was used to determine the values of  $r_S$  for seven biradicals with  $9.3 \text{ Å} \leq r \leq 11.7 \text{ Å}$ .

3. Since the dipole-dipole interaction splits the ESR lines of the allowed signal of monoradicals, that is, increases the second moment of the spectrum, the value of  $r$  can be determined directly from the magnitude of the second central moment  $M_2$  of the ESR spectrum [90], which by definition is equal to

$$M_2 = \frac{\int_{-\infty}^{+\infty} (H - H_0)^2 F(H) dH}{\int_{-\infty}^{+\infty} F(H) dH}, \quad (64)$$

where  $F(H)$  gives the form of the absorption line of the spectrum as a function of the intensity of the magnetic field  $H$ , and the center of the spectrum  $H_0$  is found from the relationship

$$\int_{-\infty}^{+\infty} (H - H_0) F(H) dH = 0. \quad (65)$$

## 5. Brief Summary of Methods for Determining the Distance between the N - O Groups

In addition to the methods indicated for the measurement of  $r$  by the direct determination of the dipole-dipole splitting or from the relative intensity of the forbidden signal  $\Delta M_S = 2$ , there also exist several methods which make it possible to determine  $r$  (and  $D$ ) in those cases where the methods indicated above are inapplicable.

For convenience we shall summarize in one place the characteristic features of the known methods for determining  $r$  between N-O fragments.

The distance  $r$  between the N-O fragments of biradicals can be measured by the following methods.

1. From the magnitude of the dipole-dipole splitting  $D$  of the lines of the ESR spectrum. In this case

$$r_D = \left( \frac{3}{2} \cdot \frac{g_e \beta_e}{D} \right)^{1/3} = 30.3 (\tilde{D})^{-1/3}, \quad (62)$$

where  $\tilde{D}$  is expressed in G, and  $r$  in Å.

If the magnitude of the dipole-dipole interaction in the biradical is large ( $\tilde{D} \geq 200$  G), it is possible to neglect the anisotropy of the spectrum and to find experimentally the value of  $2\tilde{D}$ , from which it is possible to calculate  $r_D \leq 6-7$  Å.

In the case where  $7 \text{ Å} \leq r \leq 13 \text{ Å}$ , the measurement of  $\tilde{D}$  from the ESR spectra of vitrified solutions of biradicals is a complicated problem, since the production of additional lines (relative to the ESR spectrum of the monoradical) may be due not only to dipole-dipole splitting but also to spin-exchange interaction (if the magnitude of the exchange integral  $|J| \geq |A_{ii}|$ ) and the relative orientation of the principal axes of the  $\hat{g}$  and  $\hat{A}$  tensors of the N-O fragments.

The calculation of the form of the ESR spectrum of biradicals in vitreous solutions, carried out in [115], showed that in the case where  $|J| \ll |A_{ii}|$ , for spectra in which it is possible to distinguish an intense central doublet of lines, the splitting of the lines of this doublet can with sufficient accuracy be taken as equal to  $\tilde{D}$  (see Fig. 24).

2. From the relative intensity of the lines of the ESR spectrum of the "forbidden" transitions,† corresponding to  $\Delta M_S = 2$  ( $g \approx 4$ ) [100, 103]

$$\alpha = \frac{I_2}{I_1} = \frac{8}{15} \left( \frac{3g_e \beta_e}{2H_0 r_s^3} \right)^2 \approx \frac{38}{r_s^6}.$$

Here,  $I_1$  and  $I_2$  are the integral intensities of the spectra of the corresponding transitions,  $H_0 \approx 3200$  G is the intensity of the constant magnetic field, and  $r_s$  is expressed in Å.

† The authors of the present review do not know of any studies in which  $D$  (and  $r$ ) for nitroxide biradicals have been determined from the position of the signal  $\Delta M_S = 2$  (Eq. (59)).

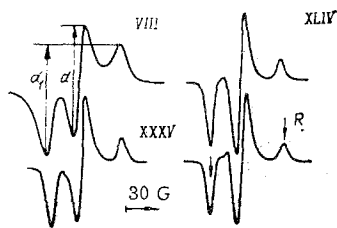


Fig. 28

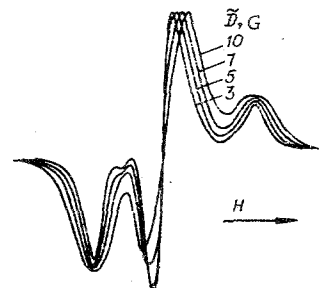


Fig. 29

Fig. 28. ESR spectra of solutions vitrified at 77°K: methanol solution of the biradical VIII and toluene solutions of the biradicals XXXV and XLIV and the monoradical 2,2',6,6'-tetramethyl-4-hydroxypiperidine-1-oxyl (R).

Fig. 29. Dependence of the form of the ESR spectra of vitrified solutions of nitroxide biradicals on the value of  $D$  for the case where the relative orientation of the radical fragments is a random function with an equal-probability distribution. For all the spectra,  $J = 0$ ,  $(1/T_2)_0 = 4$  G.

In [117] it was shown that the contribution to  $M_2$  due to dipole-dipole interaction between the unpaired electrons ( $\Delta M_2$ ) in a polycrystalline specimen is directly related to the distance  $r_M$  between them:

$$\Delta M_2 = M_2 - M_2(0) = \frac{3}{5} g_e^2 \beta_e^2 S(S+1) r_M^{-6} \quad (66)$$

or

$$r_M = 23.1(\Delta M_2)^{-1/6}. \quad (67)$$

Here,  $M_2(0)$  is the second moment of the spectrum of the monoradical in the absence of dipole-dipole interaction,  $r_M$  is expressed in Å, and  $\Delta M_2$  in  $G^2$ . This relationship is applicable for ESR spectra of any form and makes it possible to measure  $r \leq 15-16$  Å (see for example [61, 118]).

It should be noted that Eq. (66) does not take account of the possibility that when there is exchange interaction the second moment of the spectrum is not measured completely, since for large  $|J|$  ( $|J| \gg |A_{ii}|$ ) the spectrum contains widely separated components of low intensity, which are usually not recorded by the instrument, so that they "carry away" part of the second moment. Various questions associated with this problem are discussed in [119, 120]. Nevertheless, the use of Eq. (67) is fully applicable even for an unknown value of  $J$ , since the value of  $r_M$  calculated from Eq. (27) depends very little on the error in the measurement of  $\Delta M_2$ .

It has been established for biradicals of different structure that all the above methods of determining  $r$  give the same values of  $r$  within the limits of error of the measurement, so that any of them can be used (Table 8). The fact that it is impossible to measure distances  $r < 15-16$  Å, however, imposes a significant restriction on the methods listed above, and prevents their extensive application for the study of macromolecules.

4. In [121] it was shown that at distances  $r > 12$  Å the biradicals have three-component ESR spectra (Fig. 28), characteristic of monoradicals, and that for many biradicals the magnitude of the empirical parameter  $d_1/d$  differs considerably from  $(d_1/d)_0$ , characterizing dilute solutions of monoradicals even when within the limits of accuracy of the measurements the second moment  $M_2$  does not differ from that for the monoradical  $M_2(0)$ .

The same authors [121] measured and compared the values of  $d_1/d$  and the distances  $r$ , calculated from the values of  $M_2$ , for nine nitroxide biradicals vitrified in different matrices: toluene, aliphatic alcohols, and a 50% water-glycerol mixture. The empirical dependence of  $r$  on the parameter  $\Delta = (d_1/d) - (d_1/d)_0$  was obtained, described by the relationship [121]

$$r_d = 9.3 + 0.77/\Delta, \quad (68)$$

where for biradicals of the piperidine series with the "chair" conformation of the ring

$$\Delta = d_1/d + 0.036 A_{\parallel} - 1.76 A_{\parallel} \quad \text{in } G, \quad r_d \quad \text{in } \text{Å}. \quad (69)$$

In [121] it was shown that Eqs. (28) and (29) can be extrapolated for the determination of  $r$  in the range from 13 to 30 Å.

The form of the ESR spectra of nitroxide biradicals with values of  $r$  from 14 to 21 Å has also been calculated [122] (Fig. 29) for the case where the relative orientation of their N-O groups is a random parameter. The model adopted for these calculations of biradical spectra is apparently applicable to some extent for the description of the vitrified solutions of biradicals which are not very long but which are flexible - the fact that their polycrystalline ESR spectra do not contain characteristic biradical lines indicates that several conformations are stabilized simultaneously in the glasses (probably configurations with fairly close values of  $r$ ). The results of the treatment of the theoretical spectra by means of relationship (68) confirms that the extrapolation of this relationship to  $r > 16$  Å, carried out in [121] is correct.

Thus the methods at present available make it possible, for dilute vitrified solutions, to determine with a high accuracy the distance between the N-O fragments in biradicals in the range  $4 \text{ Å} \leq r \leq 30 \text{ Å}$ . As an illustration, Table 8 gives the values of  $r$  for 18 biradicals determined by different methods. An interesting feature is that in those cases where it is possible to make a comparison, the values of  $r$  measured in vitrified solutions agree within the limits of the error in the measurements with the results of the x-ray diffraction study of single crystals of the same biradicals.

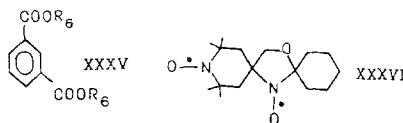
IV. APPLICATION OF BIRADICALS  
AS SPIN PROBES

In conclusion we can examine the use of nitroxide biradicals for the study of specific biological systems. The literature on this topic is not yet very extensive, but it illustrates the extensive possibilities provided by biradical SP and paired spin labels (see also the review [10]).

The studies known to the authors of the present review can be divided into three groups. Firstly, biradicals have been used as sensitive probes for the study of biological and model membranes [124-129]. The second group consists of studies in which biradical molecules have been used as specific inhibitors of the active centers of enzymes [130-132]. In the third group of studies, the authors cited have combined two spin labels in one macromolecule, and subsequently studied spin exchange or dipole-dipole interaction in this "biradical" [117, 118, 133, 134, 139].

The importance of the use of biradicals or molecular probes is based on the fact that the characteristic features of their solvation, rotational diffusion, and intermolecular spin exchange can be readily studied experimentally.

The paramagnetic N-O fragments of nitroxide biradicals in solutions with different polarity are generally solvated by the solvent molecules in the same way as the monoradicals with the corresponding structure of the piperidine or pyrrolidine ring. As an illustration, Fig. 30 compares the dependence of the HFI constants  $a$  and  $A_{\parallel}$  on the nature of the solvent (toluene, octanol, ethanol, methanol, and  $H_2O$ ) for the monoradical 2,2',6,6'-tetramethyl-4-hydroxypiperidine-1-oxyl and the biradicals I, V, XX, XXXII, and XXXV.



The values of  $a$  were measured at 25°C, and the values of  $A_{\parallel}$  at 77°K. The fact that the HFI constants  $a$  and  $A_{\parallel}$  for the mono- and biradicals in identical solvents are the same makes it possible, by measuring  $a$  or  $A_{\parallel}$ , to investigate the localization of a biradical probe in heterophase systems in the same way as when monoradicals are used [135].

The possibility of the exact determination of the correlation time of rotational diffusion  $\tau_C$  for nitroxide biradicals was analyzed in [124] for the case of the biradical XXXVI (methods for determining  $\tau_C$  by means of this biradical are reviewed in [10]).

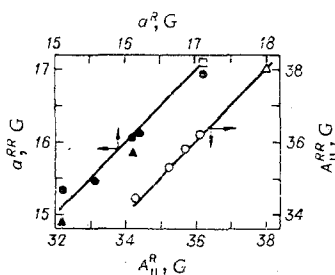


Fig. 30

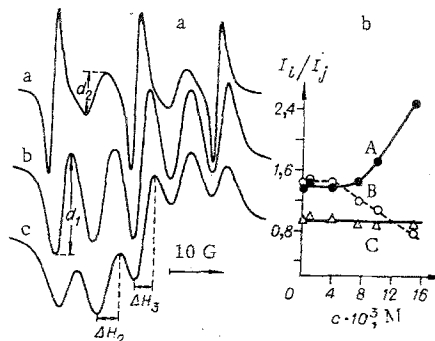


Fig. 31

Fig. 30. Dependence of the values of  $a$  (at 25°C) and  $A_{\parallel}$  (at 77°K) for nitroxide mono- and biradicals on the nature of the solvent. Monoradical (R) 2,2',6,6'-tetramethyl-4-hydroxypiperidine-1-oxyl; biradicals (RR): ●) XXXII, ○) XXXV, ▲) I, △) V, □) XX.

Fig. 31. a) ESR spectra of the biradical V at 28°C and concentrations: a)  $10^{-4}$ , b)  $7.5 \times 10^{-3}$ , c)  $1.5 \times 10^{-2}$  M. Solvent: 2% ethanol + 98% toluene (by volume). b) Dependence of the relative intensity of the components of the ESR spectrum of the biradical V on its concentration: A)  $I_2/I_1$ , B)  $I_2/I_3$ , C)  $(I_2 + I_4)/(I_1 + I_3 + I_5)$ .

It has been suggested that biradicals of type XXXVI be used as SP for the study of membranes, since the large value of  $D=230$  G ( $E=18$  G  $\ll \tilde{D}$ ) is appreciably greater than the anisotropy of the g factor and the HFI tensor, and this facilitates the analysis of the changes in the ESR spectra of biradical XXXVI. The authors cited examined the change in the form of the spectrum of biradical XXXVI with change in  $\eta/T$  and put forward empirical calibrations which make it possible to measure  $\tau_C$  for the biradical over a wide range of times  $4 \cdot 10^{-11} < \tau_C < 10^{-7}$  sec.

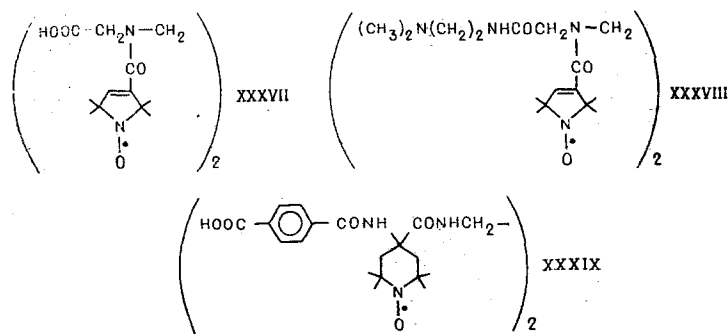
A study has been made [125] of the dynamic structure of the micelles formed by sodium dodecyl sulfate (NaDS) in aqueous solutions, using biradical XX with slow exchange (see p. 124). It was found that with increase in the concentration of NaDS above the critical concentration of micelle formation a decrease in the magnitude of the exchange integral J was observed, and the broadening of the "basic" lines indicated a decrease in the rate of rotational motion to values of the order of  $\tau_C \sim 10^{-9}$  sec. It was shown that the exchange of the molecules of biradical XX between the micelles in the aqueous phase of the solution takes place extremely slowly, with a frequency  $\nu \leq 10^7$  Hz. The process of aggregation of the molecules of the biradical to form micelles with increase in the concentration of biradical XX in the solution above  $10^{-4}$  M was studied. The effects of the interaction of the biradicals with one another must be taken into account in the study of the membranes in other heterophase systems, since neglect of intermolecular interactions may lead to erroneous conclusions.

It is possible in principle to distinguish the contributions of intramolecular and intermolecular exchange in the ESR spectrum of a biradical [140]. We can demonstrate this for the case of solutions of biradical V. A typical change in the form of its ESR spectrum with change in concentration is shown in Fig. 31a. It can be seen from Fig. 31 that increase in concentration leads to a marked change in the relative intensity of the "exchange" and "basic" components of the spectrum. This relationship is represented quantitatively in Fig. 31b, where

$$\frac{I_i}{I_h} = \frac{d_i (\Delta H_i)^2}{d_h (\Delta H_h)^2}, \quad (69)$$

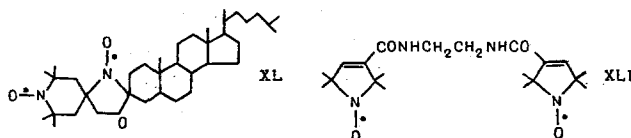
$d_i$  and  $d_h$  are the intensities, and  $\Delta H_i$  and  $\Delta H_h$  the widths of the corresponding components (see Fig. 31a). The fact that the ratio  $(I_1 + I_4)/(I_1 + I_3 + I_5)$  is independent of concentration leads to the important conclusions that the intramolecular spin exchange taking place on the collision of the N-O fragments of the same biradical is not influenced by collisions between the paramagnetic centers of different molecules (that is, intermolecular exchange), that is, it is in fact possible to distinguish these two processes experimentally, and the concentration broadening of the central component of the spectrum makes it possible to determine the magnitude of the rate constant of intermolecular spin exchange  $k_e$ .

The influence of the ionic strength and pH of the solution on intramolecular spin exchange has been studied [141] for the biradicals



containing the ionizable groups  $-\text{COO}^-$  or  $-\text{NH}^+(\text{CH}_3)_2$ . The changes in the ESR spectra are discussed within the framework of the model of rapid exchange and it is suggested that biradicals of this kind can be used for the study of pH-dependent conformational changes in membranes.

A new highly anisotropic biradical spin label XL, analogous to XXXVI, has been proposed as a sensitive probe for the study of the structure of membranes [114]:

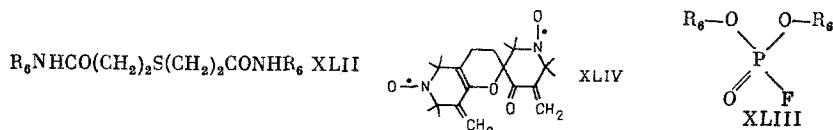


The value  $2\tilde{D} \approx 455$  G was used to calculate the distance  $r$  between the N-O groups, equal to 5 Å, and the geometry of biradical XL was refined: The piperidine ring in this biradical has the "twist" form. The study of synthetic dipalmitoyllecithin membranes spin-labeled with the biradical XL showed that their use as a probe is more effective than the use of monoradicals for the investigation of the orientation and molecular organization of membranes, since the anisotropy  $\tilde{D} \sim 200$  G is much greater than the anisotropy of the HFI (about 25 G).

A number of authors [126-128] have recorded structural transformations in membranes from the changes in the ESR spectra, associated with a change in the intramolecular exchange interaction in biradicals.

In [126] a study was made of the change in the form of the ESR spectrum of the biradical XLI in different solvents and the outer membranes of nerve cells. Unfortunately, the authors cited did not achieve their main aim, which was to record the conformational transitions in the membrane as an excitation potential passed through the nerve. The explanation was that the probe was situated in regions which did not undergo conformational changes, namely in the hydrophobic regions of the lipid fraction of the membrane.

The conformational transitions in mitochondrial membranes have been studied [127]. Changes in the ESR spectra of the probe XLII were produced by adding oxidizing substrates (succinate) and were decreased by adding inhibitors of oxidative phosphorylation ( $Zn^{2+}$  ions). Unfortunately, the calculation of the kinetic characteristics - "frequencies" of spin exchange was not carried out correctly, so that only the qualitative interpretation of the results is of interest. The same can be said of [128], which describes a study of the conformational changes in membrane  $Na^+$ ,  $K^+$ -dependent ATPase.



A study of the conformational rearrangements in a photoreceptor membrane by means of the biradical XLIV has been undertaken [129]. The subsequent development of this work appears extremely promising.

The next group of papers [130-132] described the use of the biradical XLIII, which is a specific inhibitor of esterases containing a serine residue in the active center of the enzymes. The probe XLIII combines with the OH group of the serine and makes it possible to obtain information on the structure of the active center and to measure the values of the inhibition constants. The following enzymes were studied:  $\alpha$ -chymotrypsin [130-132], cholinesterase [130, 131], trypsin, elastase, thrombin, and various subtilisins [131]. Attention was drawn not only to the change in the mobility of the probe as a whole (and the relationship between the degree of retardation of the biradical XLIII and the possible structure of the active center), but also to the change in the intensity and form of the "exchange" components. Although a quantitative analysis of these effects was not carried out, it was concluded in [130] that they are highly sensitive to the geometry of the active centers, since a definite relative orientation of the paramagnetic fragments is necessary for intramolecular spin exchange to take place.

The last group of papers [117, 118, 133, 134, 139] described the paired labeling of different amino acid groups in proteins and in enzymes by monoradical spin labels. In the absence of intermolecular interaction between the macromolecules, this system is the analog of a biradical. Measurement of the distances between the labels makes it possible to characterize quantitatively the geometry of the macromolecule and the changes which it undergoes in various rearrangements [117, 118, 133, 134], and the observation of spin exchange between the pair of labels makes it possible to reach conclusions regarding the dynamics of intramolecular motion in biopolymers [133, 139].

Thus in [118] the distance between labels attached to the SH- $\beta$ -93 group and the FG-4 histidine residue of human hemoglobin was measured, and a decrease of 3.5 Å in this distance was observed during the thermal denaturation of the protein. In [117] an estimate was made of the distances between different functional groups in lysozyme (histidine, lysines), myoglobin (histidines), and myosin (SH groups). An important feature is that the results obtained for hemoglobin show satisfactory agreement with x-ray structural models of these proteins [136-138].

In [133] the cyclic decapeptide gramicidin S was studied by labeling the two ornithine residues in the molecule with a cyanuric chloride spin label and hence obtaining an analog of a biradical. At a temperature above +30°C, spin exchange between the N-O fragments involving collisions was observed. The study of the



temperature dependence of the relative intensity of the "exchange" components, and measurement of the distances between the N-O groups in ethanol and chloroform, made it possible to reach conclusions regarding the spatial arrangement of the side chains of the modified ornithine residues in these solvents. These data show good agreement with the existing model of gramicidin S.

In [139], measurement of the broadening of the biradical components of the ESR spectrum of the doubly spin-labeled dinucleotide of uridylic acid was used to find the frequencies of the characteristic intramolecular motion.

Spin-labeled nucleosides have been synthesized by using an appropriate nitroxide spin label to acylate the 2'(3')-OH groups of ribose and the phosphate group in adenosine monophosphate (AMP) and guanidine mono- and diphosphates. Spin exchange in aqueous solutions of a biradical derivative of AMP has been studied [142].

The determination of the geometry of a macromolecule by means of the paired attachment of spin labels has been most fully and systematically used for the investigation of the conformational state of the biologically active linear nonapeptide bradykinin [134]. Circular dichroism,  $^{13}\text{C}$  NMR, and potentiometric titration data were used to carry out a theoretical conformational analysis of the bradykinin molecule. The four most energetically favorable structures of this hormone were determined. By attaching two labels to different amino acid groups, the authors cited [134] obtained six different "biradical" derivatives. By comparing the experimentally measured distances between the labels with the results of the structural conformational analysis, it was possible to establish the single conformation of the biradical formed in solution.

## V. CONCLUSION

The data given in the present review clearly indicate that the ESR spectra of nitroxide biradicals are extremely sensitive to even small changes in the conformational state of the molecules of the biradicals. By studying the characteristic features of spin exchange and the dipole-dipole interaction between the paramagnetic centers in biradicals, it is possible to extract definite information on their structure, the dynamics and thermodynamics of conformational transitions, the delocalization of spin density (electronic "conductivity") along chains of bonds in the molecule, and the possibility of collisions (and hence chemical reactions) between groups separated in space in the same molecule. The use of biradicals as spin probes and labels makes it possible to detect fine changes in biologically important systems and to obtain unique information on their intimate mechanisms. The subsequent extensive application of biradicals in these studies will probably provide the answers to many unsolved problems in molecular biology.

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## LITERATURE CITED

1. H. McConnell and B. G. McFarland, *Quart. Rev. Biophys.*, **3**, 91 (1970).
2. G. I. Likhtenshtein, *The Method of Spin Labeling in Molecular Biology* [in Russian], Nauka, Moscow (1974).
3. V. K. Kol'tover, *Progress in Science, VINITI, Biophysics* [in Russian], Vol. 4 (1975).
4. A. L. Buchachenko and A. M. Vasserman, *Stable Radicals* [in Russian], Khimiya, Moscow (1973).
5. A. N. Kuznetsov, *The Spin Probe Method* [in Russian], Nauka, Moscow (1976).
6. K. I. Zamaraev, Yu. N. Molin, and K. M. Salikhov, *Spin Exchange. Theory and Physicochemical Applications* [in Russian], Nauka, Novosibirsk (1977).
7. L. Berliner (editor), *Spin Labelling*, New York (1976).
8. H. A. Resing and C. G. Wade (editors), *Magnetic Resonance in Colloid and Interface Science*, Washington, D. C. (1976).
9. B. J. Gaffney and H. M. McConnell, *J. Magn. Res.*, **16**, 1 (1974).
10. G. R. Luckhurst, "Biradicals as spin probes," in: *Spin Labelling* (ed. by L. Berliner), New York (1976).
11. A. L. Kovarskij, A. M. Wasserman, and A. L. Buchachenko, *J. Magn. Res.*, **7**, 225 (1972).
12. R. F. Khairutdinov and K. I. Zamaraev, *Izv. Akad. Nauk SSSR, Ser. Khim.*, 1524 (1970).
13. A. I. Kokorin and K. I. Zamaraev, *Zh. Fiz. Khim.*, **46**, 2914 (1972).
14. É. G. Rozantsev, *Free Iminoxyl Radicals* [in Russian], Khimiya, Moscow (1970).
15. G. M. Zhidomirov and A. L. Buchachenko, *Zh. Strukt. Khim.*, **8**, 1110 (1967).
16. A. Hudson, "Triplets and biradicals," in: *Electron Spin Resonance* (ed. by N. M. Atherton), Vol. 1, Halsted Press, (1973), p. 69.
17. A. Nakajima, *Bussei*, **14**, 374 (1973).
18. V. N. Parmon, *Summary of Candidate's Thesis* [in Russian], Moscow Physicotechnical Institute, Moscow (1975).

19. V. A. Venderskii, V. I. Gol'danskii, A. I. Rakoed, P. A. Stunzhas, and V. A. Sokolov, *Dokl. Akad. Nauk SSSR*, 204, 1143 (1972).
20. V. F. Yudanov, K. M. Salikhov, G. M. Zhidomirov, and Yu. D. Tsvetkov, *Teor. Éksp. Khim.*, 5, 663 (1969).
21. R. A. Sadykov, R. Z. Sagdeev, Yu. N. Molin, and L. V. Volodarskii, *Izv. Sib. Otd. Akad. Nauk SSSR*, 1, No. 2, 63 (1974).
22. V. I. Muromtsev, O. N. Safronov, V. V. Pomortsev, and V. P. Golikov, *Fiz. Tverd. Tela*, 13, 2708 (1971).
23. O. L. Closs and O. E. Doubleday, *J. Am. Chem. Soc.*, 94, 9240 (1972).
24. A. Nakajima, *Bull. Chem. Soc. Jpn.*, 46, 779 (1973).
25. A. Nakajima, *Bull. Chem. Soc. Jpn.*, 46, 1129 (1973).
26. A. V. Zvarykina, V. B. Stryukov, D. N. Fedutin, and A. B. Shapiro, *Pis'ma Zh. Éksp. Teor. Fiz.*, 19, 3 (1974).
27. A. V. Zvarykina, V. B. Stryukov, S. Ya. Umanskii, D. N. Fedutin, R. P. Shibaeva, and É. G. Rozantsev, *Dokl. Akad. Nauk SSSR*, 216, 1091 (1974).
28. R. Dupeyre, A. Rassat, and J. Ronzaud, *J. Am. Chem. Soc.*, 96, 6559 (1974).
29. O. Rohde, S. P. Van, W. R. Kester, and O. H. Griffith, *J. Am. Chem. Soc.*, 96, 5311 (1974).
30. H. Lemaire, *J. Chim. Phys.*, 64, 559 (1967).
31. G. R. Luckhurst and É. G. Rozantsev, *Izv. Akad. Nauk SSSR, Ser. Khim.*, 1708 (1968).
32. H. R. Falle, G. R. Luckhurst, H. Lemaire, Y. Marshall, A. Rassat, and P. Rey, *Mol. Phys.*, 11, 49 (1966).
33. C. Corvaja, G. Giacometti, K. D. Kopple, and Zia ud Din, *J. Am. Chem. Soc.*, 92, 3919 (1970).
34. G. R. Luckhurst, *Usp. Khim.*, 40, 572 (1971).
35. G. R. Luckhurst, R. Poupko, and C. Zannoni, *Mol. Phys.*, 30, 499 (1975).
36. D. C. Reitz and S. I. Weissman, *J. Chem. Phys.*, 33, 700 (1960).
37. L. D. Landau and E. M. Lifshits, *Course of Theoretical Physics* [in Russian], Vol. 3, Fizmatgiz, Moscow (1963).
38. A. Nakajima, H. Ohya-Nishiguchi, and Y. Deguchi, *Bull. Chem. Soc. Jpn.*, 45, 713 (1972).
39. A. Nakajima, H. Nishiguchi, and G. Deguchi, *J. Phys. Soc. Jpn.*, 24, 1175 (1968).
40. V. B. Stryukov, D. N. Fedutin, and A. V. Zvarykina, *Pis'ma Zh. Éksp. Teor. Fiz.*, 19, 687 (1974).
41. A. V. Zvarykina and V. B. Stryukov, *Zh. Strukt. Khim.*, 14, 737 (1973).
42. Yu. V. Yablokov, V. V. Gavrilov, and M. N. Romanenko, in: *Radiospectroscopy* [in Russian], Nauka, Moscow (1973).
43. Y. Iida, *Bull. Chem. Soc. Jpn.*, 46, 320 (1973).
44. B. Bleaney and D. K. Bowers, *Proc. Roy. Soc., Ser. A*, 214, 451 (1952).
45. V. N. Parmon and G. M. Zhidomirov, *Mol. Phys.*, 32, 613 (1976).
46. V. N. Parmon and G. M. Zhidomirov, *Mol. Phys.*, 27, 367 (1974).
47. G. R. Luckhurst, *Mol. Phys.*, 10, 543 (1966).
48. S. H. Glarum and J. H. Marshall, *J. Chem. Phys.*, 47, 1374 (1967).
49. G. R. Luckhurst and G. F. Pedulli, *Mol. Phys.*, 18, 425 (1970).
50. G. R. Luckhurst and G. F. Pedulli, *J. Am. Chem. Soc.*, 92, 4738 (1970).
51. A. G. Redfield, *IBM J. Res. Dev.*, 1, 19 (1957).
52. J. A. Pople, W. G. Schneider, and H. J. Bernstein, *High-Resolution Nuclear Magnetic Resonance*, McGraw-Hill, New York (1959).
53. A. Carrington and A. D. McLachlan, *Introduction to Magnetic Resonance: With Applications to Chemistry and Chemical Physics*, Harper and Row, New York (1967) [Russian translation: Mir, Moscow (1970)].
54. C. S. Johnson, *Mol. Phys.*, 12, 25 (1967).
55. K. M. Salikhov, Yu. N. Molin, and K. I. Zamaraev, *J. Magn. Res.*, 5, 189 (1971).
56. V. B. Stryukov, S. Ya. Umansky, and A. V. Zvarykina, *Chem. Phys. Lett.*, 10, 240 (1973).
57. V. G. Dashevskii, *Conformation of Organic Molecules* [in Russian], Khimiya, Moscow (1974).
58. A. I. Kokorin and V. N. Parmon, *Izv. Akad. Nauk SSSR, Ser. Khim.* (1976).
59. V. N. Parmon, A. I. Kokorin, G. M. Zhidomirov, and K. I. Zamaraev, *Mol. Phys.*, 26, 1565 (1973).
60. Ya. S. Lebedev, D. M. Chernikova, N. N. Tikhomirova, and V. V. Voevodskii, *Atlas of ESR Spectra* [in Russian], Izd-vo Akad. Nauk SSSR, Moscow (1962).
61. A. I. Kokorin, V. N. Parmon, V. I. Suskina, Yu. A. Ivanov, É. G. Rozantsev, and K. I. Zamaraev, *Zh. Fiz. Khim.*, 48, 953 (1974).
62. A. Rassat, *Pure Appl. Chem.*, 25, 623 (1971).
63. A. L. Buchachenko, V. A. Golubev, A. A. Medzhidov, and É. G. Rozantsev, *Teor. Éksp. Khim.*, 1, 249 (1965).
64. A. B. Shapiro, K. Baimagambetov, M. G. Gol'dfel'd, and É. G. Rozantsev, *Zh. Org. Khim.*, 8, 2263 (1972).

65. A. B. Shapiro, V. I. Suskina, V. V. Fedorova, and É. G. Rozantsev, *Izv. Akad. Nauk SSSR, Ser. Khim.*, 694 (1970).
66. É. G. Rozantsev, V. A. Golubev, M. B. Neiman, and Yu. V. Kokhanov, *Izv. Akad. Nauk SSSR, Ser. Khim.*, 572 (1965).
67. A. L. Buchachenko, L. V. Ruban, and É. G. Rozantsev, in: *Radiospectroscopic and Quantum-Mechanical Methods in Structural Studies [in Russian]*, Nauka, Moscow (1967), p. 119.
68. V. N. Parmon, A. I. Kokorin, G. M. Zhidomirov, and K. I. Zamaraev, *Mol. Phys.* (1975).
69. É. G. Rozantsev, G. F. Pavelko, A. L. Buchachenko, and M. B. Neiman, *Izv. Akad. Nauk SSSR*, 2306 (1967).
70. E. K. Metzner, L. Libertini, and M. Calvin, *J. Am. Chem. Soc.*, **96**, 6515 (1974).
71. B. S. Tsukerblatt, M. P. Chobanu, M. I. Belinskii, and A. V. Ablov, *Fiz. Tverd. Tela*, **18**, 961 (1976).
72. A. Hudson and G. R. Luckhurst, *Mol. Phys.*, **13**, 409 (1967).
73. G. M. Nedlin, *Fiz. Tverd. Tela*, **15**, 3048 (1973).
74. P. V. Schastnev and K. M. Salikhov, *Teor. Éksp. Khim.*, **9**, 291 (1973).
75. I. A. Sysoeva, A. L. Buchachenko, A. M. Vasserman, I. S. Vasileiskaya, and D. V. Muslin, *Dokl. Akad. Nauk SSSR*, **192**, 1291 (1970).
76. R. Briere, R. M. Dupeyre, H. Lemaire, C. Morat, A. Rassat, and P. Rey, *Bull. Chem. Soc. France*, 3290 (1965).
77. A. B. Shapiro, M. G. Gol'dfel'd, and É. G. Rozantsev, *Izv. Akad. Nauk SSSR, Ser. Khim.*, 960 (1973).
78. A. B. Shapiro, M. G. Goldfield, and E. G. Rosantsev, *Tetrahedron Lett.*, 2183 (1973).
79. A. B. Shapiro, G. A. Novozhilova, and É. G. Rozantsev, *Izv. Akad. Nauk SSSR, Ser. Khim.*, 452 (1976).
80. A. B. Shapiro, G. A. Novozhilova, V. A. Dombrovskii, L. B. Volodarskii, and É. G. Rozantsev, *Izv. Akad. Nauk SSSR, Ser. Khim.*, 2124 (1976).
81. V. N. Parmon, A. I. Kokorin, and K. I. Zamaraev, *Izv. Akad. Nauk SSSR, Ser. Khim.*, No. 9 (1976).
82. V. C. Nowak, G. Kothe, and H. Zimmermann, *Ber. Bunsenges. Gesellsch.*, **78**, 265 (1974).
83. G. Kothe, J. Brickmann, and H. Zimmermann, *Adv. Mol. Relaxation Processes*, **5**, 277 (1973).
84. J. Brickmann and G. Kothe, *J. Chem. Phys.*, **59**, 2807 (1973).
85. G. Kothe, E. Ohmes, J. Brickmann, and H. Zimmermann, *Angew. Chem. (Internat. Ed.)*, **10**, 938 (1971).
86. K. Reibisch, G. Kothe, and J. Brickmann, *Chem. Phys. Lett.*, **17**, 86 (1972).
87. M. Chikira, H. Yokoi, and T. Isobe, *Bull. Chem. Soc. Jpn.*, **47**, 2208 (1974).
88. E. Wassermann, L. C. Snyder, and W. A. Yager, *J. Chem. Phys.*, **41**, 1763 (1964).
89. M. Gouterman and W. Moffitt, *J. Chem. Phys.*, **30**, 1107 (1959).
90. A. Abragam, *Principles of Nuclear Magnetism*, Oxford Univ. Press, New York (1961) [Russian translation: IL, Moscow (1963)].
91. S. N. Dobryakov, G. G. Lazarev, Ya. S. Lebedev, and M. V. Serdobov, *Zh. Strukt. Khim.*, **17**, 814 (1976).
92. A. Calder, A. R. Forrester, P. G. James, and G. R. Luckhurst, *J. Am. Chem. Soc.*, **91**, 3724 (1969).
93. V. A. Gaponenko, L. V. Mosina, and Yu. V. Yablokov, *Zh. Strukt. Khim.*, **14**, 442 (1973).
94. Z. Ciecierska-Tworek, S. P. Van, and O. H. Griffith, *J. Mol. Struct.*, **16**, 139 (1973).
95. C. A. Hutchison, Jr., and B. W. Mangum, *J. Chem. Phys.*, **34**, 908 (1961).
96. S. H. Glarum and J. H. Marshall, *J. Chem. Phys.*, **62**, 956 (1975).
97. S. N. Dobryakov, G. G. Lazarev, Ya. S. Lebedev, and M. V. Serdobov, *Zh. Strukt. Khim.*, **17**, 814 (1976).
98. S. N. Dobryakov, G. G. Lazarev, Ya. S. Lebedev, and M. V. Serdobov, *Izv. Akad. Nauk SSSR, Ser. Khim* (1976).
99. J. Kurita, *J. Chem. Soc. Jpn.*, **85**, 833 (1964).
100. Ya. S. Lebedev and V. I. Muromtsev, *ESR and the Relaxation of Stabilized Radicals [in Russian]*, Khimiya, Moscow (1972).
101. P. Kottis and R. Lefebvre, *J. Chem. Phys.*, **39**, 393 (1963).
102. P. Michon and A. Rassat, *J. Am. Chem. Soc.*, **97**, 696 (1975).
103. A. A. Dubinskii, O. Ya. Grinberg, A. A. Tabachnik, A. B. Shapiro, V. P. Ivanov, É. G. Rozantsev, and Ya. S. Lebedev, *Biofizika*, **19**, 840 (1974).
104. R. P. Shibaeva, *Zh. Strukt. Khim.*, **16**, 330 (1975).
105. O. H. Griffith, D. W. Cornell, and H. M. McConnell, *J. Chem. Phys.*, **43**, 2909 (1965).
106. W. L. Hubbell and H. M. McConnell, *Proc. Nat. Acad. Sci. U.S.A.*, **64**, 20 (1969).
107. B. G. McFarland and H. M. McConnell, *Proc. Nat. Acad. Sci. U.S.A.*, **68**, 1274 (1971).
108. P. Jost, L. J. Libertini, V. C. Hebert, and O. H. Griffith, *J. Mol. Biol.*, **59**, 77 (1971).
109. D. Bordeaux, J. Lajzerowicz-Bonneteau, R. Briere, H. Lemaire, and A. Rassat, *Org. Magn. Res.*, **5**, 47 (1973).
110. L. I. Antsiferova, N. S. Nazemets, and N. V. Rabinkina, *Mol. Phys.*, **25**, 909 (1973).

111. G. B. Birrell, S. P. Van, and O. H. Griffith, *J. Am. Chem. Soc.*, 95, 2451 (1973).
112. M. P. Eastman and J. H. Freed, *J. Chem. Phys.*, 51, 2690 (1969).
113. T. Toda, E. Mori, and K. Murayama, *Bull. Chem. Soc. Jpn.*, 45, 1764 (1972).
114. J. F. W. Keana and R. J. Dinerstein, *J. Am. Chem. Soc.*, 93, 2808 (1971).
115. V. N. Parmon, A. I. Kokorin, and G. M. Zhidomirov, *J. Magn. Res.* (1977).
116. L. D. Arutyunyan and R. P. Shibaeva, *Dokl. Akad. Nauk SSSR*, 215, 881 (1974).
117. A. V. Kulikov, G. I. Likhtenshtein, É. G. Rozantsev, V. I. Suskina, and A. B. Shapiro, *Biofizika*, 17, 42 (1972).
118. A. I. Kokorin, K. I. Zamaraev, G. L. Grigoryan, V. P. Ivanov, and É. G. Rozantsev, *Biofizika*, 17, 34 (1972).
119. L. A. Shul'man, *Ukr. Fiz. Zh.*, 18, 404 (1973).
120. L. A. Shul'man and G. A. Podzarej, *Fiz. Tverd. Tela*, 14, 1770 (1972).
121. A. I. Kokorin, Author's Abstract of Candidate's Dissertation, Institute of Chemical Physics, Academy of Sciences of the USSR, Moscow (1973).
122. V. N. Parmon and A. I. Kokorin, *Teor. Éksp. Khim.*
123. A. I. Kokorin, L. N. Bogach, A. B. Shapiro, and É. G. Rozantsev, *Izv. Akad. Nauk SSSR, Ser. Khim.*, 1994 (1976).
124. J. Michon and A. Rassat, *J. Am. Chem. Soc.*, 96, 335 (1974).
125. S. Ohnishi, T. J. R. Cyr, and H. Fukushima, *Bull. Chem. Soc. Jpn.*, 43, 673 (1970).
126. M. Calvin, H. H. Wang, G. Entine, D. Gill, P. Ferruti, M. A. Harpold, and M. P. Klein, *Proc. Nat. Acad. Sci. U. S.*, 63, 1 (1969).
127. M. Goldfield, L. Hendel, E. G. Rosantsev, A. B. Shapiro, and V. I. Suskina, *Studia Biophys.*, 20, 161 (1970).
128. L. M. Raikhman, É. P. Buzhinskii, and Yu. Sh. Moshkovskii, *Dokl. Akad. Nauk SSSR*, 212, 246 (1973).
129. S. A. Vlasova, K. E. Kruglyakova, and M. A. Ostrovskii, *Dokl. Akad. Nauk SSSR*, 223, 1001 (1975).
130. J. C. Hsia, D. J. Kosman, and L. H. Plette, *Biochem. Biophys. Res. Commun.*, 36, 75 (1969).
131. J. C. Hsia, D. J. Kosman, L. H. Plette, *Arch. Biochem. Biophys.*, 149, 441 (1972).
132. D. J. Kosman and L. H. Plette, *Arch. Biochem. Biophys.*, 149, 452 (1972).
133. V. T. Ivanov, A. I. Miroshnikov, L. G. Snezhkova, Yu. A. Ovchinnikov, A. V. Kulikov, and G. I. Likhtenshtein, *Khim. Prirodn. Soedin.*, 1, 91 (1973).
134. V. T. Ivanov, G. L. Grigoryan, et al., *Bioorg. Khim.*, 1, 1241 (1975).
135. C. Hamilton and H. M. McConnell, *Usp. Khim.*, 39, 531 (1970).
136. M. Perutz, *J. Mol. Biol.*, 13, 646 (1965).
137. C. C. F. Blake, L. N. Johnson, G. A. Mair, A. C. T. North, D. C. Phillips, and V. R. Sarma, *Proc. Roy. Soc. London Ser. B*, 167, 365 (1967).
138. I. C. Kendrew, H. C. Watson, B. E. Strandberg, R. E. Dickerson, D. C. Phillips, and V. C. Shore, *Nature*, 190, 663 (1961).
139. V. I. Leventahl, J. M. Becker, Yu. N. Molin, V. P. Kumarev, M. A. Grachev, and D. G. Knorre, *FEBS Lett.*, 24, 149 (1972).
140. N. M. Atherton, *Chem. Phys. Lett.*, 23, 454 (1973).
141. P. Ferruti, D. Gill, M. P. Kbin, and M. Calvin, *J. Am. Chem. Soc.*, 91, 7765 (1969).
142. A. I. Petrov and B. I. Sukhorukov, *Biofizika*, 20, 965 (1975).