Iminoxy Free Radicals Derived from Diethyl (1-Hydroxyimino)phosphonates. An EPR Study

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Iminoxy radicals containing the diethyl phosphonate group have been synthesized and characterized by means of EPR spectroscopy. Structures of the *Z* and *E* isomers have been established on the basis of hyperfine splitting due to ^{14}N, ^{31}P, and ^{1}H nuclei. The higher stability of the *E* isomer over the *Z* isomer has been established by analysis of anisotropic spectra. The source of a dependence of the ^{31}P hyperfine constant on the solvent polarity is discussed.

The sensitivity of the EPR parameters to solvent properties is a well recognized feature of free radicals. Analysis of changes of hyperfine parameters due to changes in the polarity of the radical environment,[1–3] as well as differences in the line width caused by motional restrictions[4–6] have been applied for many systems. The most frequent use of sterically shielded nitroxide π-radicals in such studies is justified by their high stability.

Although the iminoxy σ-radicals have been known for a long time,[7–11] they have not been used to date as spin probes. These radicals are readily obtainable by oxidation of oximes as well as nitroso and isonitroso compounds[12] or by γ-irradiation of compounds containing the C=NO–H moiety.[13,14] The relative ease of modification of remote parts of the iminoxy radicals makes them particularly attractive in cases when affinity between the spin probe and the object under study (*e.g.* cell membranes, enzymatic centres) is to be exploited for the location and immobilization of paramagnetic centres.

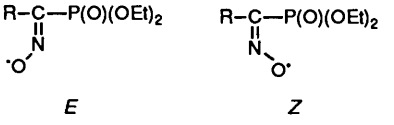
Recently[15] we reported the synthesis and EPR data for some iminoxy free radicals containing the diethyl phosphonate group. Structurally similar α-aminophosphonic acids have been proved to participate in the inhibition of several enzymatic centres.[16] The large ^{31}P hyperfine constant in the EPR spectra of these radicals seems to be a useful tool in studying radical structure and interaction with the environment.[15]

In the present paper we report the results of a study on several new phosphorus-containing radicals which are a part of our extended project concerning iminoxy free radicals and their application as spin probes.

Results and Discussion

Hyperfine Interaction Analysis.—The C–N bond of the C–N–O moiety in the iminoxy radicals possesses only partial double-bond character,[15] and thus rotation around it is restricted, but not completely frozen. Consequently, the presence of two isomers in equilibrium is observed for most of the iminoxy radicals.[7] In the case of diethyl (1-oxoiminoalkyl)-phosphonates we assigned the isomer having the oxygen of the C–N–O moiety in the *syn* position to the phosphonate group as the *Z* isomer, while in the *E* isomer both fragments are *anti* to each other.

The isomers differ in their EPR parameters and in population in solution, but in the cases of radicals **1a**–**4a** the *E* isomer always dominates, regardless of substituent R. A similar observation has been made previously[15] for radicals **5a** and **7a**. The most striking difference between the isomers is the change of the phosphorus hyperfine constant on going from *E* to *Z*. The A_P value for isomer *E* varies from 0.65 to 1.17 mT, depending on substituent R, while for the isomer *Z* the phosphorus hyperfine constant lies in the range 5.27–5.60 mT. The



E *Z*

1a; R = *o*-Tolyl
2a; R = 1-Naphthyl
3a; R = 2-Naphthyl
4a; R = Diethoxyphosphinylmethyl [$(EtO)_2(O)P–CH_2–$]
5a; R = Phenyl
6a; R = Methyl
7a; R = *tert*-Butyl

difference in A_N is much less profound (0.32–0.34 mT for *E* and 0.30–0.31 for *Z*).

While for A_P and A_N differences between the *E* and *Z* isomers are qualitatively the same for all radicals, the proton hyperfine splitting differentiates the systems under study into three categories.

Radicals **1a** and **2a** (Fig. 1) display similar, small (0.03–0.06 mT), proton hyperfine constants for both isomers. However, it is clear (see insets in Fig. 1) that the electron interacts with different sets of nuclei *i.e.* for **1a**–**Z**, three equivalent methyl protons; for **1a**–**E**, one proton; for **2a**–**Z**, one proton; and for **2a**–**E** two inequivalent protons.

For the iminoxyls **5a** and **7a**[15] A_H has been found to be equal to 0.14 and 0.11 mT, respectively, in the case of the *E* isomer, dropping to about zero for the *Z* isomer. Similarly, for the radical **3a** (Fig. 2) there is a marked difference in A_H between isomers, however in this case splitting due to two inequivalent protons is observed for both isomers (*Z*, 0.17 and 0.11 mT; *E*, 0.04 and 0.02 mT).

In radicals **4a** and **6a** protons of substituents R are at the shortest distance from the C–N–O fragment. In these cases the proton hyperfine constant is similar for both isomers and equal to *ca.* 0.1 mT. It is noteworthy that phosphorus hyperfine splitting due to the presence of another diethyl phosphonate group in the substituent R of **4a** also has a similar, or at least not drastically different, hyperfine constant for both isomers (*Z*, 0.41 mT; *E*, 0.53 mT) (Fig. 3).

The values of hyperfine constants due to particular nuclei for both isomers of the radicals **1a**–**7a** are collected in Fig. 4.

The above differences between the groups of radicals reflect differences in the steric hindrance imposed by the substituent R but also seem to be the consequence of spin-density distribution between oxygen and nitrogen of the iminoxy radicals. In these σ-radicals the unpaired electron is distributed nearly equally between the oxygen and nitrogen nuclei.[18] The hyperfine interaction of atoms other than those having major contribution to the MO containing the unpaired electron (*i.e.* C–N–O) arises partially by a dipolar mechanism. In such a case one can expect the strong dependence of hyperfine constants on

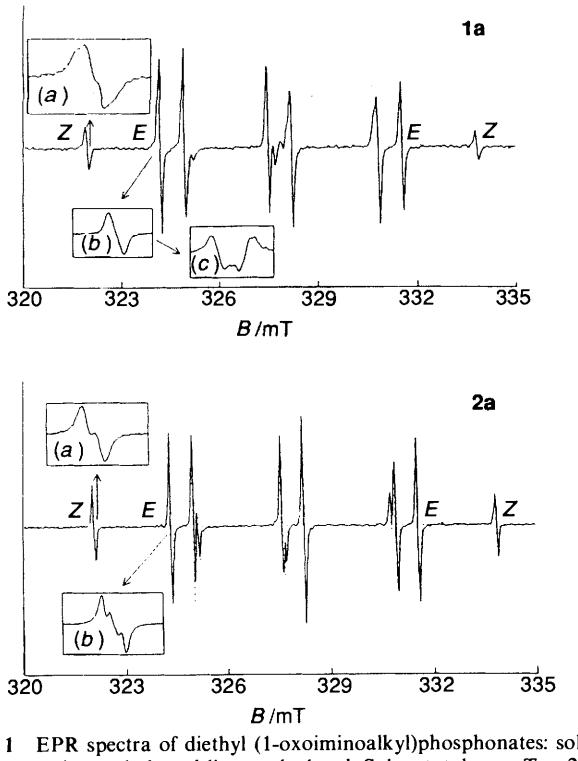


Fig. 1 EPR spectra of diethyl (1-oxoiminoalkyl)phosphonates: solid lines, experimental; dotted lines, calculated. Solvent, toluene; T = 293 K. Top, radical **1a** (R = *o*-tolyl). Bottom, radical **2a** (R = 1-Naphthyl). The spectral lines which are solely due to particular isomers Z or E are labelled Z or E, respectively. In the insets (*a*) and (*b*) the expansions of the first lines due to Z and E isomers, respectively are presented. Inset (*c*) shows the expansion of the second derivative of the first spectral line of the E isomer of **1a**.

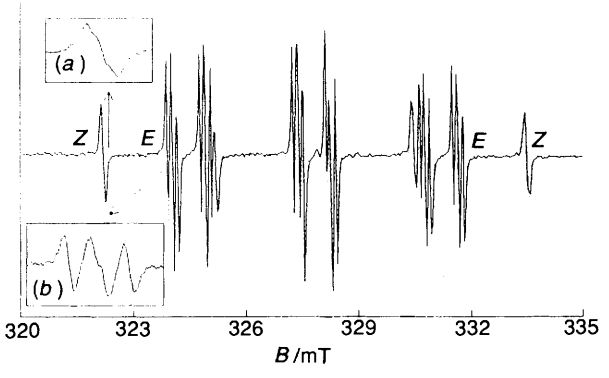


Fig. 2 EPR spectra of the radical **3a** (R = 2-Naphthyl): solid line, experimental; dotted line, calculated. Solvent, toluene; T = 293 K. The spectral lines which are solely due to isomer Z or E are labelled Z or E, respectively. In the insets (*a*) and (*b*) the expansions of the first lines due to the Z and E isomers, respectively are presented.

the distance between a particular nucleus and the paramagnetic centre. On the other hand, there are two equally populated centres of the spin density in the iminoxy radical (O and N, where the electron resides on the sp^2 lobe). Thus, the non-zero hyperfine splitting due to the particular nucleus can arise from the proximity of either oxygen or nitrogen of the iminoxy group. This accounts for the observation of phosphorus hyperfine splitting also for the E isomer. Although in this isomer the oxygen atom is pointing in the opposite direction to that of the phosphorus atom, the sp^2 hybrid of nitrogen, containing *ca.* 50% of spin density, is close enough to produce a measurable hyperfine splitting due to ^{31}P.

Molecular modelling shows a restriction in rotation around the C–C bond of the R–CNO fragment in the case of the E isomers of **1a** and **2a** due to the presence of substituents in the α-position of the aromatic ring. There is no hyperfine splitting due to methyl protons for the E isomer of **1a**, because steric overcrowding prevents the proximity of iminoxyl oxygen and the α-methyl. In the case of the Z isomer the repulsive interaction between the α substituent on R and the phosphonate group makes more favourable the position in which methyl protons can interact with the spin density on the nitrogen atom. A similar situation applies for radical **2a**.

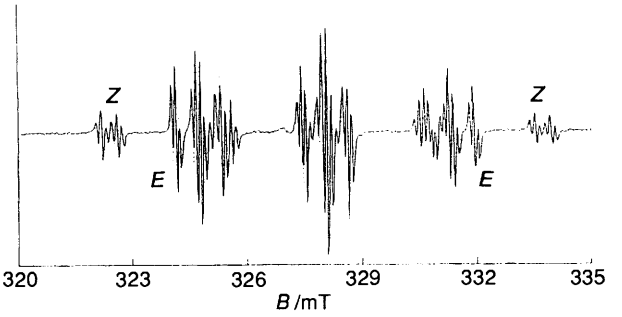


Fig. 3 EPR spectra of the radical **4a**: solid line, experimental; dotted line, calculated. Solvent, CCl_4; T = 293 K. The spectral lines which are solely due to isomer Z or E are labelled Z or E, respectively.

For radicals **3a**, **5a** and **7a** structures of the substituents R do not promote in any way the interaction of protons with nitrogen, thus there is a big difference in A_H between the E and Z isomers.

Finally, in the case of **4a** and **6a** the closeness of the methylene or methyl protons to the iminoxy group leads to observation of similar proton hyperfine constants for both isomers which are about three-times greater than in the case of **1a** or **2a**.

Anisotropic Spectra.—The ^{13}C NMR spectrum of the oxime **1** in $CDCl_3$ reveals the presence of only one isomer, which is most likely the E isomer. However, after oxidation to the **1a** radical, both E and Z isomers have been observed, thus isomerization occurs, as expected, because the C=N bond is weaker in the iminoxy radicals than in the parent oximes.[15] The γ-irradiation of a solid sample of **1** allows observation of the anisotropic spectrum of **1a**. Only the parallel component of the ^{14}N hyperfine tensor is well resolved in the spectrum giving $A_{||}$ = 3.8 mT at 77 K. The lack of the large ^{31}P hyperfine splitting (larger than 6 mT), which could be attributed to the Z isomer, indicates the absence of this isomer in the solid. Dissolution of the γ-irradiated sample in toluene resulted in the observation of the isotropic spectrum of **1a** in which signals of both isomers were present.

The iminoxy radicals have a strong tendency to dimerization,[18] which makes observation of anisotropic spectra in frozen solution difficult. Our efforts to obtain spectra of partially oriented **1a** in liquid nematic phases and clay minerals failed for the same reason. Interestingly, the spectrum obtained in the isotropic phase of the liquid crystal vanished after cooling to the nematic phase temperature, and reappeared in isotropic solution. Nevertheless, we were able to obtain a frozen solution spectrum of **5a** in toluene. This weak spectrum ($g_{||}$ = 2.0087, $g_{\perp}$ = 2.0067, $A_{||}^{N}$ = 3.84 mT) shows only the presence of the E isomer, being very similar to that obtained by γ-irradiation of solid **1**. This is in line with a previous observation that the population of the Z isomer decreases with temperature.[15]

Solvent Dependence.—Unlike the case of nitroxide radicals[1,2] and some iminoxyls,[12] there is no strong solvent dependence of the nitrogen hyperfine splitting in the case of diethyl (1-oxoiminoalkyl)phosphonates[15] which could be rationalized in terms of any solvent polarity parameter. Besides, for both isomers A^N does not change markedly on going from one

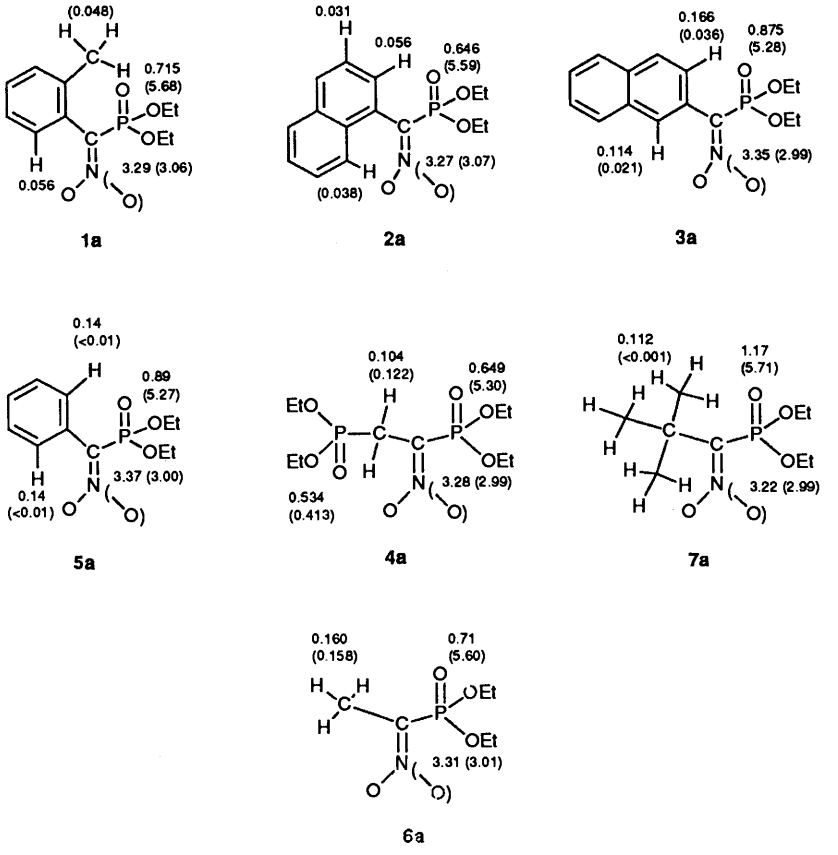


Fig. 4 Structures and hyperfine constants of radicals **1a**–**7a**. The numbers associated with particular groups or atoms represent hyperfine constants (in mT) due to particular nuclei in the following manner: $-P(O)(OEt)_2$, ^{31}P; $\geq C=N-O$, ^{14}N; $-CH_3$ or $-H$, ^1H hyperfine constant. Numbers in parentheses represent hyperfine constants for the *Z* isomer of each radical, and the symbol (–O) indicates the direction of N–O group in this isomer.

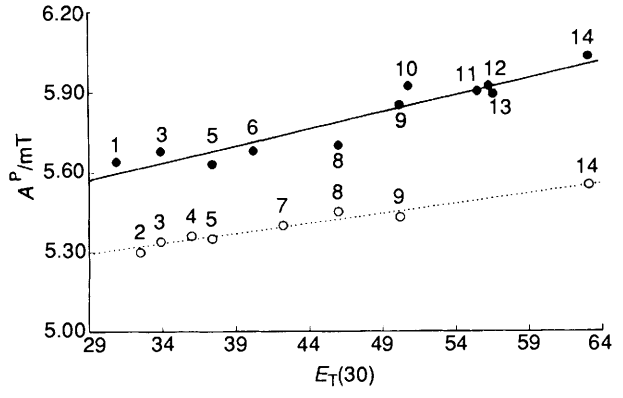


Fig. 5 Correlation of phosphorus hyperfine constant of the *Z* isomer of radicals **1a** (full circles, solid line) and **4a** (open circles, dotted line) with Dimroth-Reichardt solvent polarity parameter $E_T(30)$. Numbers associated with the points denote solvents in which measurements have been performed: 1, hexane; 2, CCl_4; 3, toluene; 4, dioxane; 5, tetrahydrofuran; 6, pyridine; 7, acetone; 8, acetonitrile; 9, butanol; 10, benzyl alcohol; 11, methanol; 12, ethylene glycol; 13, formamide; 14, water.

solvent to another, varying for example in the case of **1a** within ±0.02 mT around 3.31 mT for the *E* isomer and around 3.09 mT for the *Z* isomer. Much bigger differences have been observed for A^P of the *Z* isomer in the case of **5a**, **7a**,[15] **1a** and **4a** in various solvents. For example, the ^{31}P hyperfine constant for the *Z* isomer of **1a** varies from 5.63 mT in hexane to 6.03 mT in water. These changes can be rationalized by means of Dimroth–Reichardt solvent polarity parameters $E_T(30)$.[19,20] The A^P dependence on $E_T(30)$ for **1a** and **4a** is presented in Fig. 5. The correlation coefficients *r* from the least-squares analysis are equal to 0.94 for **1a** (11 solvents) and 0.98 for **4a** (8 solvents). The slopes are 0.0132 and 0.0075, respectively. The value of slope for **1a** is close to those obtained for **5a** and **7a** (0.0131 and 0.0162).[15] Similar correlations were performed for A^N of nitroxide radicals, always giving about three-times smaller values of slopes,[1] which may be due to the smaller absolute value of A^N (*ca.* 1.6 mT) in these π-radicals in comparison with ^{31}P hyperfine constants of *Z* isomers of oxoiminophosphonates (5.5 mT).

Although it seems that only the phosphorus hyperfine constant of the radicals under study is a well-documented solvent polarity parameter, the explanation for it is not straightforward. In the case of nitroxides, solvents modify the spin-density distribution between the nitrogen and oxygen atoms of the nitroxide fragment and hence the A^N values.[1] A similar mechanism may be taken into account in the case of some iminoxy radicals, where a dependence of A^N on solvent polarity has been found.[12] Introduction of the diethyl phosphonate group into the radical molecule makes A^N insensitive to the properties of the environment which is probably due to the enhancement of steric hindrance in the vicinity of the paramagnetic centre. The screening influence of a bulky tetrahedral substituent makes an iminoxy group inaccessible for the solvent molecules and the modification of the spin-density distribution does not occur. Under these circumstances the solvent can alter the spin density on the phosphorus nucleus only by slightly changing the distance between the oxygen of the iminoxy group and the phosphonate group, and in this way modification of the dipolar contribution to the hfs occurs.

Introduction of a second diethyl phosphonate group into the

iminoxy radical molecule **4a** causes a decrease of the A^P *vs.* $E_T(30)$ slope by a factor of *ca.* two (Fig. 5), which means that sensitivity of the phosphorus hyperfine splitting to the solvent properties is lower in this system. The ^{31}P hyperfine splitting due to the second phosphorus nucleus does not depend on the solvent polarity for either isomer. This indicates that the geminal position of the phosphorus with respect to the C=N–O group is crucial for obtaining the solvent dependence of A^P. This in turn may suggest modification of sources of spin density present on the phosphorus other than the dipolar one (*i.e.* delocalization of the electron density on the phosphorus atom and spin polarization) by the solvent. Still, geometrical changes rather than changes in the spin distribution between N and O atoms are assumed to be responsible for the phenomenon in the light of the lack of A^N solvent dependence.

Conclusions

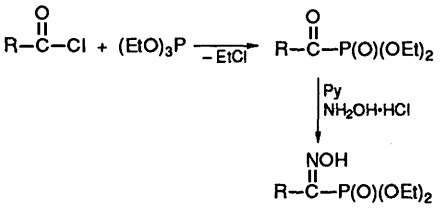
Phosphorus hyperfine structure analysis is a valuable source of structural information on the iminoxy radicals because it allows unequivocal assignment of the spectral lines to the particular isomer.

Simple method of preparation, ease of structural modification, and relative high stability[15] in solution make the phosphonate iminoxy radicals suitable for application as spin probes.

The sensitivity of the A^P parameter on the solvent properties is particularly promising, since it suggests that phosphorus-containing iminoxy radicals can be used in studies of the polarity of enzymatic centres having affinity for the phosphonate group.

Experimental

The method used for syntheses is a part of a typical synthesis of α-aminophosphonic acids.[17] The diethyl (1-hydroxyiminoalkyl) phosphonates were obtained from the appropriate acid chlorides and triethyl phosphite by the Arbuzov reaction,[21] followed by reaction of the resulting (1-oxoalkyl)phosphonates with hydroxylamine hydrochloride–pyridine in ethanol solution according to the following scheme:



The diethoxyphosphinylacetyl chloride, used for synthesis of **4**, was prepared from diethyl ethoxycarbonylmethanephosphonate (Merck) by the hydrolysis of the carboxylic ester with KOH in methanol (cleavage was monitored by ^{13}C NMR spectroscopy) followed by reaction of the resulting potassium diethoxyphosphinylacetate with oxalyl chloride in benzene. In the case of other systems the acid chlorides are commercially available or were prepared by chlorination of appropriate carboxylic acids by standard methods.

Oximes **1**, **2** and **3** are solids, sparingly soluble in water and well soluble in most of common organic solvents. **4** is a non-volatile liquid soluble both in water and in organic solvents, except saturated hydrocarbons. ^{13}C, ^{31}P and ^1H NMR spectra confirmed structures of the oximes and showed the presence of traces of benzene (the solvent used in the Arbuzov reaction and during the purification process), triethyl phosphate, and the corresponding ethyl carboxylate. The amount of impurities does not exceed 1%, according to the integration of ^1H NMR signals.

Solutions of the iminoxy radicals **1a**–**4a** for the EPR investigations were obtained by *in situ* oxidation of 0.1 mol dm^{-3} solutions of the corresponding oximes with AgO.

γ-Irradiation of the solid sample of **1** was carried out for 3 days using ^{60}Co source which is a facility of the local Radiochemical Laboratory.

EPR measurements were carried out on an SE Radiopan X-band spectrometer connected with an IBM-PC microcomputer through the locally made interface collecting 2K experimental points for each spectrum (resolution 0.007 mT). Nuclear magnetometer and EPR standards were used for calculation of *g*-factors and magnetic field calibration. The modulation frequency and amplitude were 100 kHz and 0.036 mT, respectively. The spectra were recorded at room temperature or at 77 K, using a quartz finger dewar.

The spectra were simulated using an originally written program applying an IBM-PC microcomputer. An automatic fitting procedure by the Marquardt method was used in establishing the *g*-factors, hyperfine constants, line widths, line width dependence on the M_1 spectral index and the isomer population factors.

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