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Dissociative Electron Capture of Propylene Oxide: Matrix Isolation ESR Study

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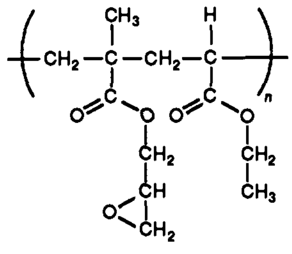
Abstract: A dissociative electron capture process of ethylene oxide yielding the anion radical of the ring-ruptured form, $O^--CH_2-CH_2^\bullet$, has been demonstrated recently. A similar electron capture process was examined for propylene oxide, 1,2-epoxybutane, and glycidyl methyl ether in an effort to determine which C–O bond would cleave, if any. The epoxy molecules of interest and Na atoms were cocondensed in argon matrices, and the electron transfer between them was effected by mild radiation ($\lambda > 580$ nm). ESR examination of the matrices revealed that, in each case, the anion had the ring-opened structure resulting from cleavage of one of the C–O bonds. The external C–O bond cleaved preferentially. A concerted, S_N2-type process is suggested between the epoxy molecule and the approaching electron. The conformations of the anion radicals were deduced from the observed α- and β-proton coupling tensors.

Introduction

Epoxylated poly(meth)acrylate such as glycidyl methacrylate–ethyl acrylate copolymer **1** is exceptionally sensitive toward electron beam erradiation, and is widely used as an electron beam negative resist in microlithography.[1,2] Recently we have shown that an epoxy system such as ehtylene oxide and trimethylene oxide undergoes, on capture of an electron, a ring-opening process illustrated below.[3]



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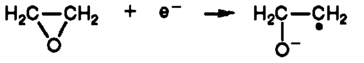

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The demonstrated dissociative electron-capture process offers an explanation for the high sensitivity of epoxylated polymers such as **1** as an electron beam negative resist.

When the epoxy moiety is structurally asymmetric as in **1**, however, a question of practical importance is which C–O bond would cleave on capture of an electron. The present report describes the result of a matrix isolation ESR study conducted to this end. Propylene oxide, 1,2-epoxybutane, and glycidyl methyl ether were cocondensed in argon matrices together with Na atoms, and electron transfer between them was induced by mild radiation ($\lambda > 580$ nm) as in the previous study.[3] In each case the resulting epoxy anion was found to have ring-ruptured structures, and it was the external C–O bond which cleaved preferentially.

Experimental Section

The liquid helium cryostat–ESR spectrometer system, which would enable trapping of vaporized metal atoms in inert gas matrices, and examination of the resulting matrices by ESR has been described earlier.[4] The technique of trapping Na atoms as electron donors and molecules of interest as acceptors, and inducing electron transfer between them by mild radiation ($\lambda > 580$ nm), thus generating cations and anions effectively isolated in inert gas matrices, has also been described.[4]

In the present series of experiments the Na atoms were generated from a resistively heated stainless steel tube (250 °C), and were trapped in argon matrices containing a controlled amount (~1–3 mol %) of epoxy molecules.

The ESR spectrometer used was an IBM Instruments Model 200D, X-band spectrometer, and the microwave frequency locked to the sample cavity was typically 9.420 GHz. For photoirradiation of the matrix, a high-pressure Xe-Hg lamp (Oriel, 1 kW unit) was used. The light beam was passed through a water filter and a broad-band (±50 nm) interference filter of choice, and was focussed on the cold finger ~40 cm away. As the metal atom concentration was ~1/10 of that of the acceptor molecules, when irradiated with yellow light ($\lambda = 600 \pm 50$ nm), the electron transfer was complete in about 4 min, as attested in bleaching of the matrix color from purple to white, and in complete disappearance of the ESR signals due to Na atoms.

Propylene oxide, 1,2-epoxybutane, and glycidyl methyl ether were all obtained from Aldrich Chemicals, and partially deuterated propylene oxide ($CD_3\overline{CHCH_2O}$) was obtained from MSD Isotopes.

Spectra and Assignments

Propylene Oxide. Figure 1 shows the ESR spectrum observed from an argon matrix in which the Na atoms and propylene oxide (1%) had been cocondensed. The spectrum is that observed prior to photoirradiation. Three widely spaced quartets, A, B, and C, and a group of closely spaced signals, X, centered about the position corresponding to $g = 2.00$ are recognized. The quartet A is due to isolated Na atoms, the quartet pattern arising from the hf (hyperfine) interaction with the ^{23}Na nucleus ($I = {}^3/_2$, natural abundance = 100%, $\mu = 2.2174\ \beta_n$). As seen in the earlier study by Jen et al., the Na atom signals are split further due to multiple trapping sites;[5] two major sites, one with the largest hf coupling constant (hence presumably with the least perturbation) and the other with the smallest coupling constant (hence with the largest perturbation), are most conspicuous.

The resonance positions and line shapes of the quartets B and C are very similar to those of the quartets seen in the previous study and assigned to Na atoms complexed with one and two epoxy molecules, respectively.[3] The B and C quartets observed here are accordingly assigned to mono- and bis(propylene oxide)–Na complexes. The doublet-like splitting of the B signals is believed to reflect the two major trapping sites seen for the isolated Na atoms.

The g values and the ^{23}Na hf coupling constants of B and C quartets were determined as follows:

for Na(propylene oxide)$_1$ $g = 1.9989$ (6)
$A(^{23}\text{Na}) = 723$ (3) MHz

for Na(propylene oxide)$_2$ $g = 1.9980$ (6)
$A(^{23}\text{Na}) = 544$ (3) MHz

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(1) Thompson, L. F.; Feit, E. D.; Heidenreich, R. D. *Polymer Eng. Sci.* **1974**, *14*, 529.

(2) Thompson, L. F.; Ballantyne, J. P.; Feit, E. D. *J. Vac. Sci. Technol.* **1975**, *12*, 1280.

(3) Kasai, P. H. *J. Am. Chem. Soc.* **1990**, *112*, 4313.

(4) Kasai, P. H. *Acc. Chem. Res.* **1971**, *4*, 329.

(5) Jen, C. K.; Bowers, E. L.; Cochran, E. L.; Foner, S. N. *Phys. Rev.* **1962**, *126*, 1749.

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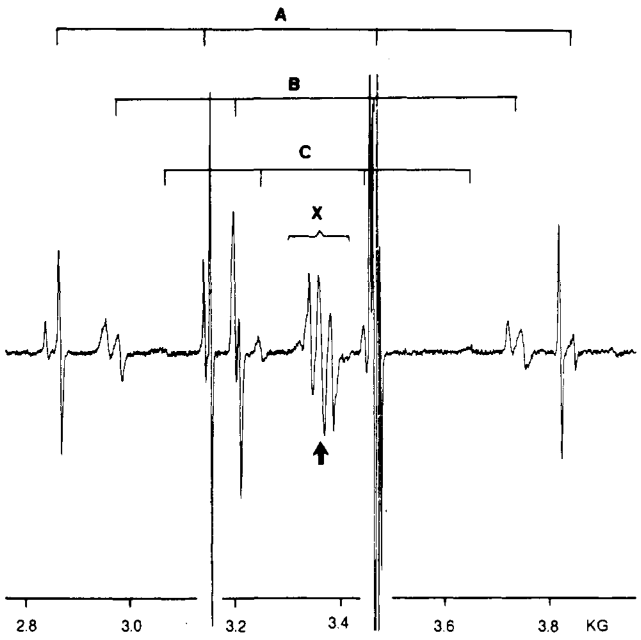

Figure 1. ESR spectrum observed from the Na/propylene oxide(1%)/Ar system prior to photoirradiation. The letters A, B, and C indicate the quartets due to isolated Na atoms and mono- and bis(propylene oxide)-Na(0) complexes, respectively. Signals X are ascribed to spontaneously formed propylene oxide anions. The arrow indicates the position corresponding to $g = 2.00$.

The values given for Na(propylene oxide)$_1$ are for the complex at the least perturbed sites. It was envisaged that these complexes were formed by the dative interaction of the oxygen lone-pair electrons with the vacant valence orbitals of Na, and the unpaired electron was in an sp-hybridized orbital pointing away from the ligand(s).[3]

The central signals X clustered around the position of $g = 2.00$ must be due to propylene oxide anions generated from spontaneous electron transfer during deposition. Such spontaneous electron transfer was observed previously during deposition of Na and ethylene oxide, but not with Na and trimethylene oxide.[3] Figure 2A shows, in an expanded scale, the central section of Figure 1 encompassing the inner two hf components of the isolated Na atoms; the letters indicate the groups to which each individual signal belongs. When the matrix was irradiated sequentially with increasingly more energetic light (800 nm → 600 nm), the quartet signals disappeared in the order of C, B of the most perturbed site, B of the least perturbed site, A of the most perturbed site, and finally A of the least perturbed site, and the X signals increased concurrently at each stage. Figures 2B and 2C show the spectra obtained after irradiation with 700-nm and 600-nm light in the sequence. The sharp quartet which emerged after irradiation with 600-nm light is due to the methyl radicals ($A = 23.3$ G). Photolysis of the propylene oxide anion must lead to formation of the methyl radicals. When the matrix was irradiated with 450-nm light, the methyl signals increased further, and the anion signals nearly disappeared (Figure 2D). No radical was formed when an argon matrix containing propylene oxide alone was irradiated with light in the range 800 → 300 nm.

Figure 3A shows, in a further expanded scale, the X region of the spectrum observed after irradiation with 700-nm light. It became apparent that the unexpectedly complex pattern seen here is due to presence of several isomers of the anions. In order to facilitate the analysis, the experiment was repeated using partially deuterated propylene oxide **2**.

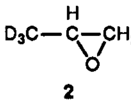

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Figure 3B shows the ESR spectrum (the X region) of the Na/methyl deuterated-propylene oxide (1%)/Ar system observed prior to photoirradiation. Deuteration of the methyl group resulted in removal of weak outer signals; the strong, triplet-like pattern in the central section remained unaffected, however. Figure 3C shows the spectrum of the same matrix observed after irradiation with 700-nm light. Clearly there are (at least) two species responsible for the central triplet-like pattern. After careful analysis of these spectra, it was concluded that the spectral pattern seen in Figure 3A was a superposition of three spectra, one due to anions **3** resulting from cleavage of the internal C–O bond, and the second and third from two conformational isomers of anions **4** resulting from cleavage of the external C–O bond.

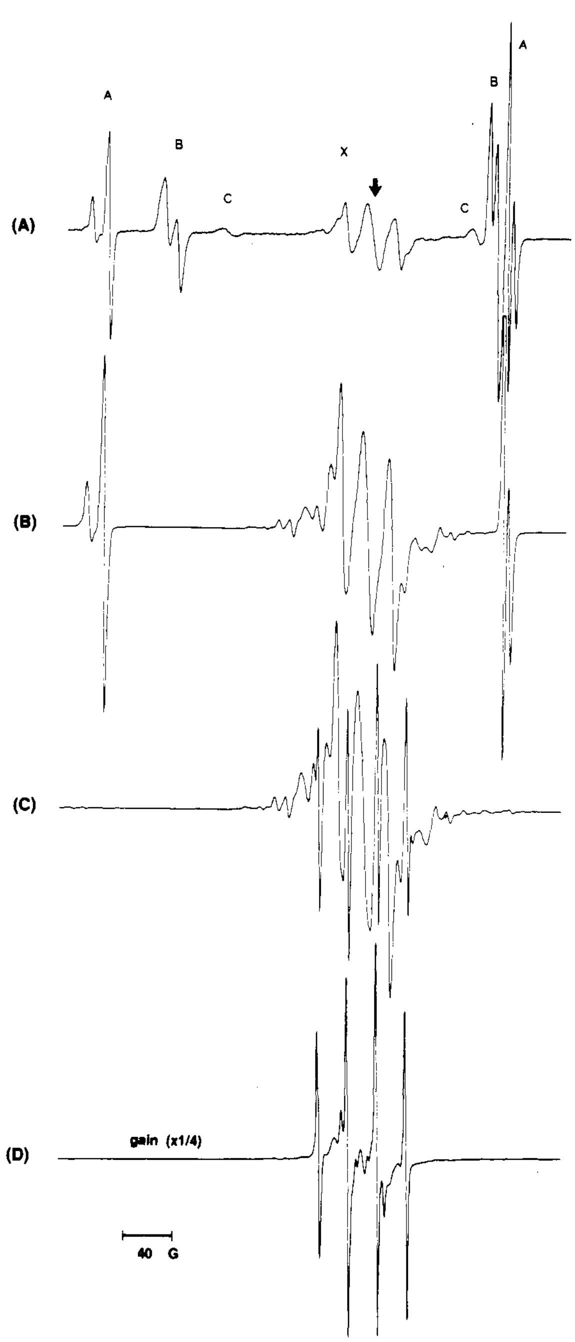
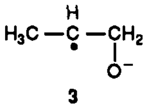
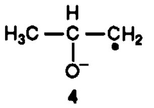


Figure 2. (A) The central section of Figure 1 shown in an expanded scale. The notations A, B, C, and X indicate the respective origins of signals (see Figure 1), and the arrow indicates the position corresponding to $g = 2.00$. (B–D) Spectra (same central section) observed after irradiation with 700-, 600-, and 450- (±50)-nm light.



$$\mathrm{H_3C-\underset{\overset{|}{O^-}}{\overset{H}{\underset{\bullet}{C}}}-CH_2}$$

3



$$\mathrm{H_3C-\underset{\overset{|}{O^-}}{\overset{H}{C}}-\underset{\bullet}{C}H_2}$$

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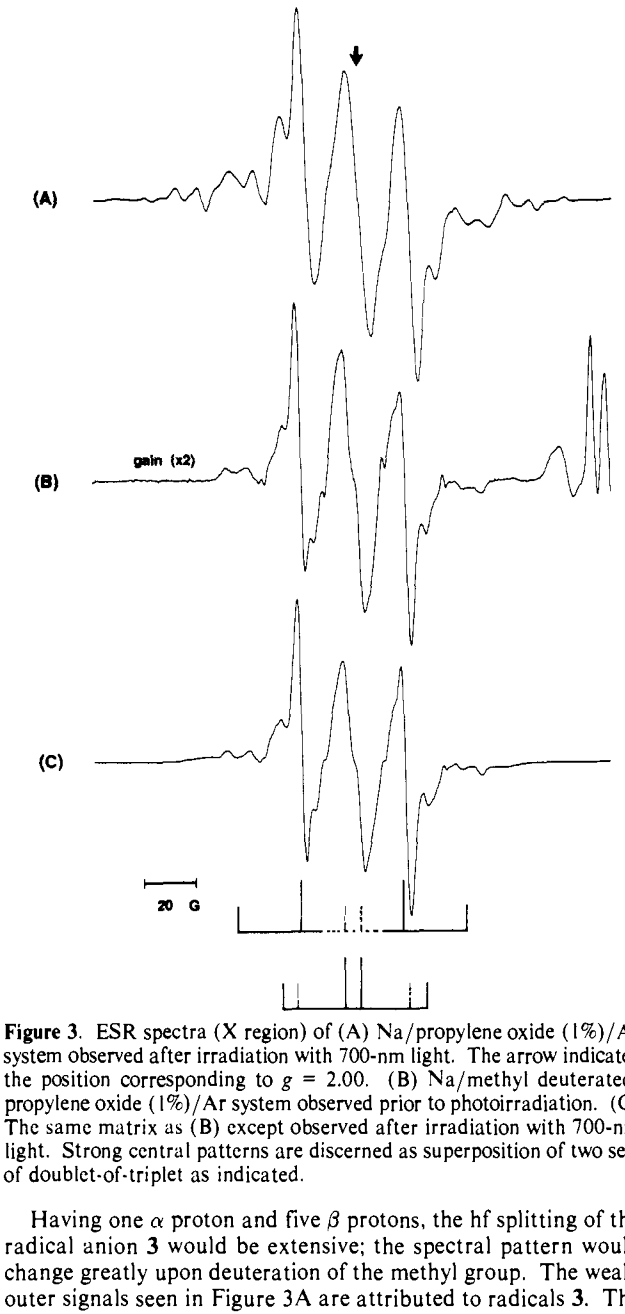
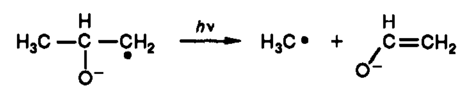


Figure 3. ESR spectra (X region) of (A) Na/propylene oxide (1%)/Ar system observed after irradiation with 700-nm light. The arrow indicates the position corresponding to $g = 2.00$. (B) Na/methyl deuterated-propylene oxide (1%)/Ar system observed prior to photoirradiation. (C) The same matrix as (B) except observed after irradiation with 700-nm light. Strong central patterns are discerned as superposition of two sets of doublet-of-triplet as indicated.

Having one α proton and five β protons, the hf splitting of the radical anion **3** would be extensive; the spectral pattern would change greatly upon deuteration of the methyl group. The weak, outer signals seen in Figure 3A are attributed to radicals **3**. The spectral pattern of the anions **4** would be a doublet (due to the β proton) of triplets (due to the two α protons), and would not be affected by deuteration of the methyl group. The strong central signals seen in Figure 3A through C, were thus discerned as superposition of two sets of doublet-of-triplet as indicated, and ascribed to the anion radicals **4** of two different conformations. The β-proton coupling constant is large (~40 G) in one conformation, and small (~5 G) in the other. Cleavage of the external C–O bond is clearly preferred. Further analysis of the spectra is presented in the Discussion section.

Closer inspection of Figure 2D (the spectrum observed after irradiation with 450-nm light) showed that the outer weak signals due to anion radicals **3** remained unscathed. The major species, anion radicals **4**, must photolyze as follows:



$$H_3C-\overset{H}{\underset{O^-}{C}}-\dot{C}H_2 \xrightarrow{h\nu} H_3C\bullet + \overset{H}{\underset{O^-}{C}}=CH_2$$

Figure 4 shows the spectral changes observed when the Na/methyl deuterated-propylene oxide (1%)/Ar system was irradiated with more energetic light (600 and 450 nm). Emergence of the CD_3 radicals (the heptet with $A = 3.67$ G) at the expense of the signals due to anion radicals is clearly revealed.

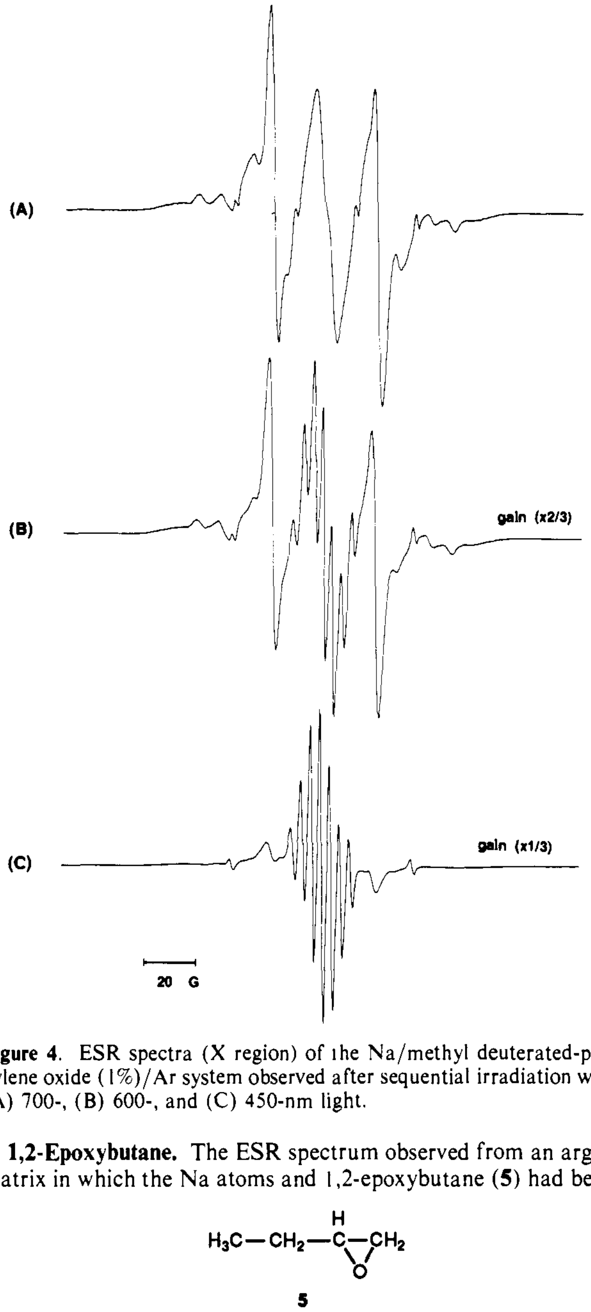
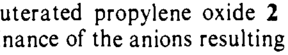


Figure 4. ESR spectra (X region) of the Na/methyl deuterated-propylene oxide (1%)/Ar system observed after sequential irradiation with (A) 700-, (B) 600-, and (C) 450-nm light.

1,2-Epoxybutane. The ESR spectrum observed from an argon matrix in which the Na atoms and 1,2-epoxybutane (**5**) had been



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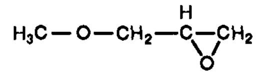
cocondensed also showed the signals due to the mono- and bis(epoxy) complexes and the X signals due to spontaneously formed anion radicals. Upon irradiation with 650-nm light, the signals due to the complexes and those of isolated Na atoms diminished and the X signals increased.

The X region of the spectrum observed after irradiation is shown in Figure 5. The spectrum is intriguingly similar to that observed from partially deuterated propylene oxide **2** (Figure 3B). It indicates the dominance of the anions resulting from cleavage of the external C–O bond, and the preponderance of the two particular conformations assumed by these anions.

Glycidyl Methyl Ether. As a model system that closely approximates the epoxy moiety of the resist system **1**, glycidyl methyl ether **6** was chosen and examined. As expected, an argon matrix



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containing the Na atoms and glycidyl methyl ether showed the ESR signals, A, B, C and X, due to the isolated Na atoms, the mono- and bis(ligand) complexes, and the spontaneously formed

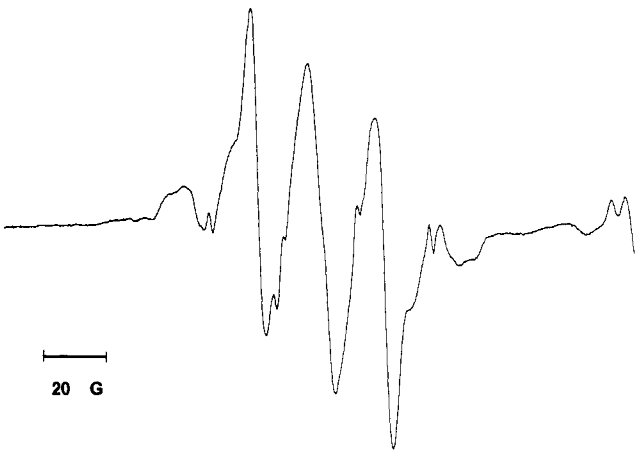

Figure 5. ESR spectrum (X region) of the Na/1,2-epoxybutane (2%)/Ar system observed after irradiation with 650-nm light.

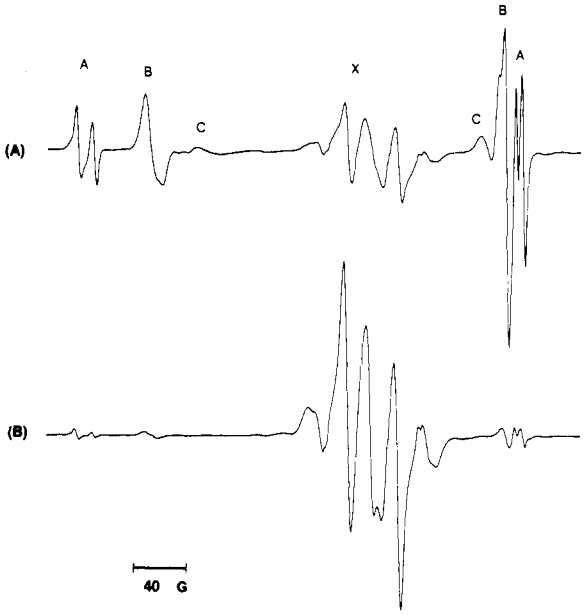

Figure 6. ESR spectra (central section) of the Na/glycidyl methyl ether (2%)/Ar system observed (A) prior to photoirradiation, and (B) after irradiation with 600-nm light. Signals due to isolated Na atoms, (A) mono- and bis(ligand)Na(0) complexes, (B and C), and anion radicals, (X) are indicated.

anion radicals, respectively (Figure 6A).

In the previous study, it was found that a noncyclic ether, di(*n*-propyl) ether, also formed mono- and bis(ligand) complexes with Na atoms.[3] Its propensity to form the mono(ligand) complex was especially strong. The Na-to-ether electron-transfer process corresponding to that observed between the Na atoms and epoxy molecules was not observed, however. Glycidyl methyl ether (**6**) thus can complex with Na with either oxygen. The B and C signals seen in Figure 6A are probably due to complexes of both types. That would account for the unusually broad line widths of these signals.

The spontaneously formed anions must be those resulting from the epoxy moiety of the molecule. The spectral pattern in the X region is very similar to that observed with 1,2-epoxybutane; the propensity of the external C–O bond (of the epoxy ring) to cleave upon an electron capture, and preponderance of the two particular conformations for the resulting anion radicals are again indicated. The β-proton coupling constants of the two conformers, 38 and 8 G, are slightly different, however, from those determined for the propylene oxide and 1,2-epoxybutane cases (40 and 5 G). Photoirradiation of the matrix with 600-nm light resulted in nearly total disappearance of the A, B, and C signals and corresponding increase of the X signals (Figure 6B).

Discussion

In the previous study, it was discussed that spontaneous electron transfer between Na atoms and ethylene molecules occurred as the LUMO (lowest unoccupied molecular orbital) of the latter was below the semifilled orbital of Na. It was noted further that the LUMO of ethylene oxide was an antibonding in-plane σ orbital confined to the C–O–C sector.[3] Similar situations must hold for propylene oxide, 1,2-epoxybutane, and glycidyl methyl ether. At any rate, the anion radicals formed through cleavage of a C–O bond in the epoxy sector of these molecules would be alkyl radicals, the spectral pattern of which are determined primarily by the hf interactions of the α and β protons.

The hyperfine coupling tensor of an α proton of an alkyl radical is well understood; it is rather anisotropic and typically assumes the following form:[6,7]

$$A_{\parallel} = 12\ \text{G},\ A_{\perp,\parallel} = 22\ \text{G},\ A_{\perp,\perp} = 35\ \text{G} \qquad (1)$$

Here $A_{\parallel}$ is the principal element of the coupling tensor measured along the C–H bond, and $A_{\perp,\parallel}$, for example, is the element measured perpendicular to the C–H bond, but parallel to the p_π orbital of the α carbon. Thus when there are two α protons, the orientations of their hf coupling tensors would differ by ~120° about the p_π orbital, and the two protons would not always be equivalent in a given magnetic field. This inequivalency would vanish, however, if the protons interchange their positions rapidly through rotation or by tunnelling. A triplet pattern consisting of a strong, sharp central component and weak, broad (~10 G wide) outer components with successive spacings of ~20 G is expected in this case.[8]

The hyperfine coupling tensor of a β proton of an alkyl radical is also well-understood; it is essentially isotropic and is given by the dihedral angle θ of the C_β–H_β bond relative to the p_π orbital.[9]

$$A(H_\beta) = B_0 + B\cos^2\theta \qquad (2)$$

It has been empirically determined that $B_0 = 4$ G, and $B = 50$ G. Thus, when there are two β protons, they would generally have different coupling constants unless the conformation is such that the two protons have the same dihedral angles.

A computer program that would simulate the ESR powder pattern of a radical having multiple sets of hyperfine coupling tensors of differing orientations has been described earlier.[7] The effect of interchanging a particular pair of protons is addressed by the method of the modified Bloch equation.[10,11]

As stated earlier, the strong central patterns seen in Figure 3, A through C, were recognized as superposition of two sets of doublet-of-triplet and were ascribed to radicals **4** of two conformations. In each set the doublet pattern is isotropic, consistent with its assignment to the β proton, and the triplet pattern is that expected from a pair of rapidly exchanging α protons.

Figures 7A and 7B are computer-simulated spectra of anion radicals **4** based on the model α-proton coupling tensor given above and the isotropic β-proton coupling constant of 40 G and 5 G, respectively. Rapid exchange of α protons at the rate ≥40 MHz was assumed. Figure 7C shows the simulated spectrum assuming the presence of one part of the conformer with the β-proton coupling constant of 40 G, and two parts of the conformer with the β-proton coupling constant of 5 G. For this simulation the α-proton coupling tensor of the latter conformer was reduced by ~20% in order to attain the best agreement with the observed pattern (i.e., Figure 3B).

The β-proton coupling constants of 40 G and 5 G imply, from eq 2, the respective dihedral angles of 32° and ~80°. Figure 8

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(6) McConnell; H. M.; Strathdee, J. *Mol. Phys.* **1959**, *2*, 129.

(7) Kasai, P. H. *J. Am. Chem. Soc.* **1972**, *94*, 5950.

(8) See ref 3 for the dependency of the spectral pattern upon the rate of exchange of the two α protons in $RCH_2^{\bullet}$.

(9) Morton, J. R. *Chem. Rev.* **1964**, *64*, 453.

(10) See, for example: Martin, M. L.; Martin, G. J.; Delpuech, J. *Practical NMR Spectroscopy*; Heyden: London, 1980; pp 293–311.

(11) An example of the modified Bloch equation method applied to an ESR powder pattern is given by: Schlick, S.; Kevan, L. *J. Am. Chem. Soc.* **1980**, *102*, 4622.

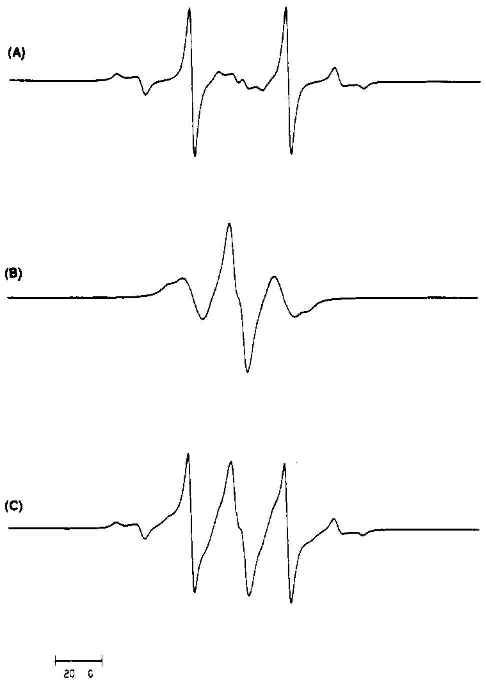

Figure 7. ESR powder pattern simulated for anion radicals **4** using the model α-coupling tensor given in the text and (A) assuming the β-proton coupling constant of 40 G, (B) assuming the β-proton coupling constant of 5 G, and (C) superposing (A) and (B) with the weighting factor of 1:2. Rapid exchange of the α protons at the rate ≥40 MHz was assumed.

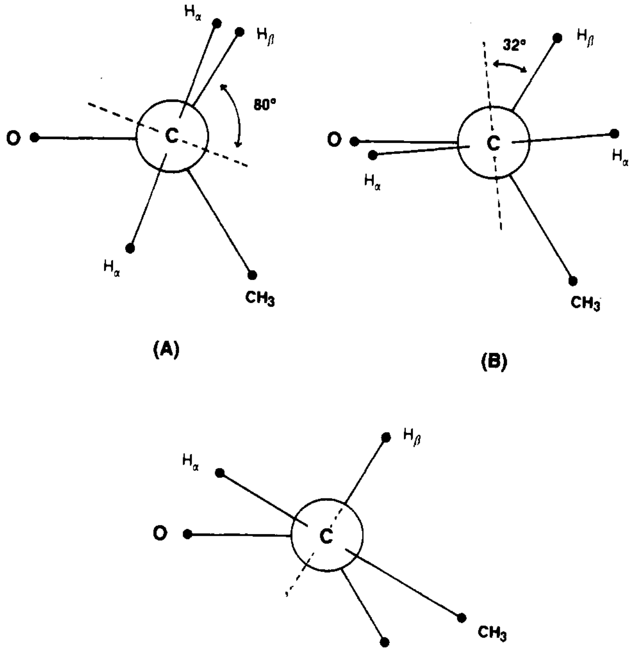

Figure 8. (A and B) Conformations (Newman diagrams) of anion radicals **4** with the small (5 G) and large (40 G) β-proton coupling constants, respectively. (C) Conformation of anion radicals **3**.

shows the Newman diagrams of the most likely conformations of radicals **4** consistent with these dihedral angles. No apparent conversion of one conformer to another was noted during many photoirradiation sequences performed on the Na/propylene oxide/argon system. The conformer with the smaller β-proton coupling constant (Figure 8A) was preferentially formed in the spontaneous electron-transfer process which occurred during deposition without irradiation. The conformer with the larger β-proton coupling constant (Figure 8B) was formed preferentially when the anions were produced via photoinduced electron transfer. The former conformation may be that dictated by the interaction with the Na$^+$ cation present in its vicinity. The indicated reduction by ~20% of the unpaired electron density at the α carbon may be ascribed to the presence of a weak σ orbital interaction between the α carbon and oxygen atom. The unpaired electron is then in the antibonding orbital of that sector. The second conformation must be that of isolated anions **4**; a hydrogen bonding is likely responsible for the energy trough. Exactly the same conformation was found for the ethylene oxide anion.[3]

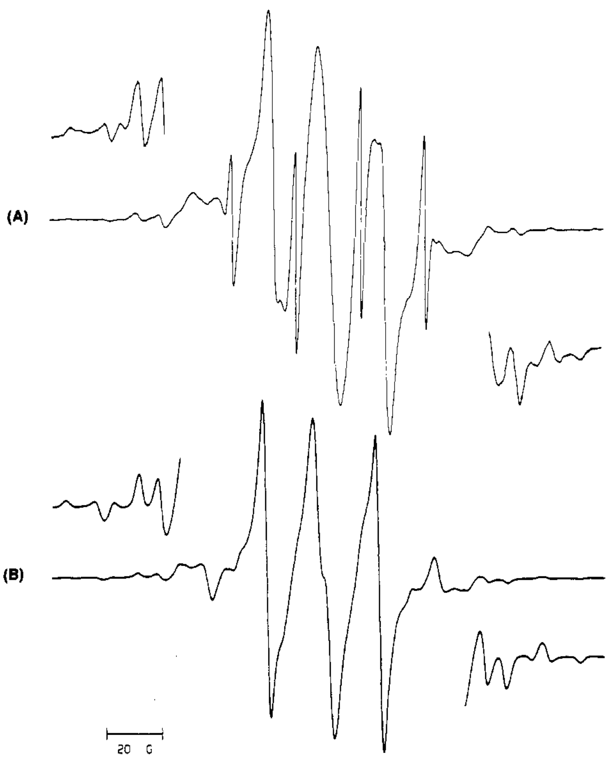

Figure 9. (A) ESR spectrum (X region) of the Na/propylene oxide (2%)/Ar system observed after irradiation with 600-nm light for 4 min. The outer sections where only anion radicals **3** contribute are observed with higher (×4) instrument gain. (B) ESR spectrum simulated for a mixture of radicals **4** of large β-proton coupling constant, radicals **4** of small β-proton coupling constant, and radicals **3** with the respective abundance ratio of 1.0/2.0/0.3. The outer sections are shown expanded vertically.

Anion radicals **3**, recognized only by the weak, outer signals (cf. Figure 3A), were produced mostly by the photoinduced process. It is reasonable to assume that the methyl group in the anion radical **3** is rapidly rotating; the three protons in the methyl group would then have the same coupling tensor. The two protons in the methylene group are likely to have unequal coupling constants. Though the coupling tensor of a β proton is essentially isotropic as given by eq 2, small anisotropies have been observed for alkyl radicals generated in crystals or those trapped in inert matrices.[9,12,13] The anisotropy is typically +2 G along the C_α–C_β bond, and -1 G perpendicular to it. When there are as many β protons as in the present case, the effect of these anisotropies are cumulative and significant for the outer components. The following β-proton coupling tensors combined with the α-proton coupling tensor given by eq 1 were found to give the best fit with the observed outer components. For the methyl protons $A_\parallel = 27$ G, $A_\perp = 24$ G, and for the methylene protons $A_\parallel = 56$ G, $A_\perp = 53$ G for one proton and $A_\parallel = 18$ G, $A_\perp = 15$ G for the second proton. It was taken into consideration that the orientations of these tensors relative to the α-proton coupling tensor differ by

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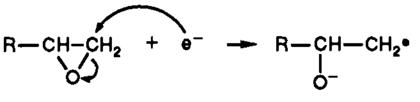
(12) McDowell, C. A.; Raghunathan, P.; Shimokoshi, K. *J. Chem. Phys.* **1973**, *58*, 114.

(13) Adrian, F. J.; Cochran, E. L.; Bowers, V. A. *J. Chem. Phys.* **1973**, *59*, 3946.

±120°, respectively, about the p_π orbital. Figure 8C shows the conformation of the anion radical **3** indicated by the methylene proton coupling constants.

Figure 9A shows the ESR spectrum (the X region) obtained when the Na/propylene oxide (2%)/Ar system deposited with the window covered was subsequently irradiated with 600-nm light for 4 min. The outer sections of the spectrum where only anion radicals **3** contribute were examined with higher instrument gain (×4) as shown. Figure 9B is the spectrum simulated for a mixture of the two conformers of anion radicals **4** and anion radicals **3** elaborated above. The abundance ratio of 1.0/2.0/0.3 for radicals **4** of large β-proton coupling constant, radicals **4** of small β-proton coupling constant, and radicals 3, respectively, was assumed. The outer sections of the simulated spectrum were also shown expanded vertically. Based on these analyses we conclude that propylene oxide, on capture of an electron, undergoes a ring-opening process where the external C–O bond is cleaved preferentially (~90%).

Experimental results obtained for 1,2-epoxybutane (Figure 5) and glycidyl methyl ether (Figure 6) indicate that the probability for the cleavage of the internal C–O bond diminishes further with increasing size of substituent at the β carbon. Ring opening of small cyclic ethers (ethylene oxide and trimethylene oxide) by nucleophilic reagents is well known.[14] When the system is unsymmetrically substituted, the nucleophilic attack occurs at the primary carbon in the majority of cases and is ascribed to a S_N 2-type concerted process. The observed dissociative electron capture process of the epoxy system requires little atomic displacement, but some change in the disposition of valence electrons. It is probable that, influenced by the electron-donating property of the alkyl substituent, the electron capture and ring-opening process occur in a concerted manner as depicted below.



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[14] See, for example: Roberts, J. D.; Caserio, M. *Basic Principles of Organic Chemistry*; Benjamin: Reading, MA, 1977; pp 659-665.

Proton NMR Study of the Heme Rotational Mobility in Myoglobin: The Role of Propionate Salt Bridges in Anchoring the Heme

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Abstract: The ^{1}H NMR spectra of the metcyano complexes of the reconstruction products with sperm whale apomyoglobin with the four coprohemin-type isomers, members of the series (propionate)$_n$(methyl)$_{8-n}$porphine-iron(III) with $n = 4$ with one propionate on each pyrrole, have been recorded and analyzed. Three of the coprophemins reconstituted cleanly at acidic pH to yield holoproteins with molecular/electronic structures minimally perturbed from those of the native protein; coprohemin IV failed to exhibit detectable incorporation into the heme pocket. Nuclear Overhauser effect spectra provided the unique orientation of the three coprohemins in the pocket, with the nonnative protonated propionates preferring to replace one or both vinyls and tolerating the replacement of one, but not two, interior methyl groups within the folded holoprotein. At alkaline pH, deprotonation of one of the interior propionates leads to spontaneous dissociation into hemin and apomyoglobin. The heme methyls of centrosymmetric coprohemin I and II complexes of sperm whale metMbCN, as well as the sperm whale and horse myoglobin complexes of centrosymmetric dipropionate, hexamethyl hemin, exhibited temperature-dependent saturation transfer among symmetry-related hemin methyls in the holoprotein that can be traced to rotational "hopping" of the hemin about an intact iron–His F8 bond. Measurement of the saturation factors and selective relaxation rates yields hopping rates of 1–10 s^{-1} at ambient temperatures. Quantitative analysis of variable temperature data for the coprohemin II complex yielded $E_a \sim 17$ kcal/mol and $A \sim 6 \times 10^{12}$ or $\Delta H^* \sim 17$ kcal/mol, $\Delta S \sim 0$, indicating that the reorientation takes place within the folded holoprotein. The rate of rotational hopping of the dipropionate hemin was found ~3 times faster in horse than sperm whale metMbCN, indicating that the propionate residue CD3 salt bridge is more stable for the Arg CD3 in sperm whale than the Lys CD3 in horse myoglobin, which supports previous conclusions reached on the basis of labile proton exchange and preferential formation of propionate salt bridges in the two proteins.

Introduction

The strong binding of the prosthetic group to the polypeptide chain of myoglobin (Mb) involves a number of interactions that contribute to the stability of the folded holoprotein. These include the bond between the proximal His F8 imidazole and the iron, salt bridges between the protein and the two heme propionates, and strong hydrophobic or van der Waals interactions between the large heme π system and the hydrophobic side chains that predominantly line the pocket.[1-3] These latter interactions appear to provide the dominant stabilization, as witnessed by the stable and structurally minimally perturbed proteins found when either the iron is removed from the heme center[4,5] or one or both of the salt bridges are abolished.[6,7] Even large aromatic dyes bind relatively strongly in the heme pocket.[8] In spite of the tight binding of the heme, the Mb pocket exhibits remarkable flexibility

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