ESR and ENDOR Studies of Cation Radicals Derived from Benzodipyran Compounds. Structure and Dynamic Behavior of Heterocyclic Ring in Tocopherol (Vitamin E)

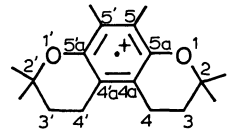
Kazuo MUKAI,* Yuichi UEMOTO, and Kazuhiko ISHIZU
Department of Chemistry, Faculty of Science, Ehime University, Matsuyama 790
(Received February 8, 1985)

ESR and ENDOR spectra of cation radicals of benzodipyran compounds were measured in CH_2Cl_2, and the proton hyperfine coupling constants were correctly determined for each radical. From detailed analysis of the temperature dependence of hyperfine coupling of β- and γ-methylene protons in the heterocyclic ring, the equilibrium conformation of the β-methylene group and activation energy for the conformational interconversion have been determined. The structure of the heterocyclic ring was compared with those of α-tocopherol (vitamin E) model previously obtained by X-ray analysis. The result suggests that the solution and crystal conformations of the heterocyclic ring are distinctly different.

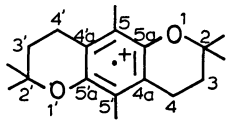
It is well recognized that tocopherols (vitamin E) are localized in cellular membranes and have function as an antioxidant by protecting unsaturated lipids from peroxidation.[1,2] The ESR studies of the "tocopheroxyl" neutral radicals (6-chromanyloxyl radicals from tocopherol) are biologically interesting since they are involved in antioxidant action of vitamin E. Therefore, in recent years, several investigators including present authors have studied the ESR spectra of α-, β-, γ-, and δ-tocopheroxyl radicals and their model compounds in detail.[3-9] The proton hyperfine coupling constants were determined correctly, and the methyl-substitution effects on the unpaired spin distribution and radical stability of the tocopheroxyl radicals have been discussed.[7,9]

Recently, Ingold *et al.* have reported absolute rate constants, k_1, for the reaction of α-tocopherol and some related phenols (for example, 4-methoxy-2,3,5,6-tetramethylphenol) with peroxyl radicals (ROO·).[11-14] By comparing the k_1 value for α-tocopherol with those found for structually related phenols that lacked the 6-membered heterocyclic ring, they suggested that the structure of this ring was largely responsible for the high reactivity of α-tocopherol. In a previous paper, from the results of the ENDOR measurements of α-tocopheroxyl and its model, we reported that each of the two β- and γ-methylene protons in the heterocyclic ring shows an equivalent hyperfine coupling (see Fig. 1).[6] This suggests that the two carbon atoms C-4 and C-3 in the heterocyclic ring are coplanar with the aromatic ring. The result is inconsistent with the X-ray structure of α-tocopherol model, which shows that the dihedral angle, θ_γ, between the C_3–C_4 bond and the aromatic ring is 11.1 and 11.9°.[11,13] However, in a previous work, the clear ENDOR spectra of the α-tocopheroxyl and its model radicals were observable in the comparatively high and narrow temperature range (from −25 to −35 °C for α-tocopheroxyl and from −40 to −55 °C for α-tocopheroxyl model), because these radicals are not so stable and dimerize at low temperature region.

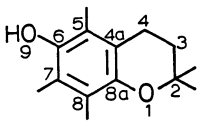
In the present paper, we have succeeded in measuring ESR and ENDOR spectra of cation radicals obtained by the oxidation of benzo[1,2-*b*:4,3-*b'*] and [1,2-*b*:4,5-*b'*]dipyran compounds **1** and **2** (see Fig. 1), which have heterocyclic ring common to that of tocopherols, in $AlCl_3$–CH_2Cl_2 mixtures. The proton hyperfine coupling constants were correctly determined for each radical, and further the temperature dependence of the hyperfine coupling constants has been studied in detail. From the results, the electronic structure of the cation radicals and the conformation and dynamic behavior of the heterocyclic ring have been discussed.



(1)



(2)



α -Toc. model

Fig. 1. Molecular structures of benzo[1,2-*b*:4,3-*b'*] and [1,2-*b*:4,5-*b'*]dipyran cation radicals **1** and **2** and α-tocopherol model, and the atomic numbering system.

Experimental

Preparation of Specimens. 2,3- and 2,5-dimethylhydroquinones were prepared according to the method of Nilsson *et al.*[15] Benzo[1,2-*b*:4,3-*b'*] and [1,2-*b*:4,5-*b'*]dipyran compounds (**1**; mp 101—102 °C and **2**; mp 196—197 °C) were synthesized by condensation of 2-methyl-3-buten-2-ol to the corresponding alkylhydroquinone, according to the method of Nilsson *et al.*[16]

Measurements. The ESR measurements were carried out using a JES-FE-2XG spectrometer equipped with a Takeda-Riken microwave frequency counter. The *g*-values were measured relative to the value of Li-TCNQ powder, calibrated with $(KSO_3)_2NO$ (g=2.0054).[17] The ENDOR spectra were recorded by a JES-EDX-1 spectrometer, operated with 80 Hz magnetic field modulation. All the ESR and ENDOR spectra have been measured in a sealed, degassed system.

Results and Discussion

ESR and ENDOR Spectra of Benzodipyran Cation Radicals 1 and 2. The cation radicals **1** and **2** were prepared by the oxidation of the corresponding benzodipyran compounds in an $AlCl_3$–CH_2Cl_2 solution. The ESR spectra of the **1** and **2** are shown in Figs. 2 and 3, respectively. The ESR spectra are complex and exhibit line-width alternation phenomena. This is due to the hindered rotation of the heterocyclic ring in radicals **1** and **2**. The g_{iso}-values of these cation radicals observed in CH_2Cl_2 at −105 °C are given in Table 1.

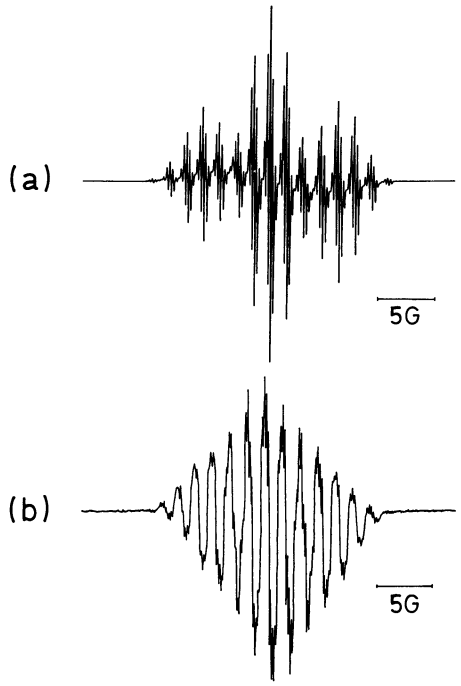


Fig. 2. ESR spectrum of cation radical **1** in CH_2Cl_2 at (a) 20 °C and (b) −100 °C.

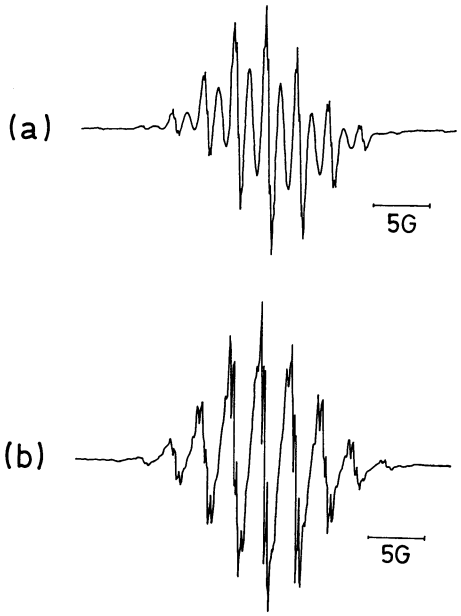


Fig. 3. ESR spectrum of cation radical **2** in CH_2Cl_2 at (a) 20 °C and (b) −100 °C.

Table 1. Hyperfine couplings (a_i^H) (in gauss) and spin densities (ρ_i) of benzodipyran cation radicals **1** and **2** in CH_2Cl_2

		$a_{5,5'}^{CH_3}$	$a_{4,4'}^{CH_2}$	$a_{3,3'}^{CH_2}$	$a_{2,2'}^{CH_3}$	T/°C	g_{iso}-values
1	ENDOR	1.547[a]	4.910, 1.547	0.285	0.046	−40	
		1.547	5.024, 1.444	0.389, 0.199	0.040[c]	−105	2.00367[d]
	ρ(Exptl)	0.0573	0.0932[b]	—	—	−105	
	ρ(Calcd)	0.0731	0.0787	—	—		
2	ENDOR	2.981	2.412	0.237	0.041	−40	
		2.986	2.452, 2.349	0.355, 0.141	0.038[c]	−105	2.00368
	ρ(Exptl)	0.1106	0.0593[b]	—	—	−105	
	ρ(Calcd)	0.0976	0.0548	—	—		

a) Experimental errors ±0.010 G. 1 G = 10^{-4} T. b) Spin densities at C-4a,4'a. c) It is not clear at present whether the smallest hyperfine coupling $a_{2,2'}^{CH_3}$ is due to two or four methyl groups at C-2,2'. d) Experimental errors ±0.00005.

ENDOR measurements of the cation radicals were performed on several samples under slightly different conditions of oxidation, varying the concentration of the benzodipyran precursors and the amount of $AlCl_3$. The ENDOR spectra of the cation radicals were observed in the broad temperature range −105 to −40 °C. Figures 4 and 5 show ENDOR spectra of cation radicals **1** and **2** in CH_2Cl_2, respectively, where the free proton frequency ν_0 (13.8 MHz) is shown by an arrowhead in the diagram.

The ENDOR spectrum of **1** clearly shows six different proton hyperfine splittings at −105 °C, as shown in Fig. 4(b). The spectrum changed explicitly by increasing temperature, and showed four different hyperfine splittings at −55 °C (see Fig. 4(a)). The cation radical **2** also shows notable temperature dependence of the ENDOR spectrum. The temperature dependence of the hyperfine couplings observed for the cation radicals **1** and **2** is shown in Figs. 6 and 7, respectively, and the hyperfine coupling constants obtained at −105 and −40 °C are summarized in Table 1.

If all the proton hyperfine couplings due to 5-CH_3, 4-CH_2, 3-CH_2, 2-$(CH_3)_2$ are different from each other, we can expect seven different hyperfine couplings for both the cation radicals **1** and **2**, as a maximum, assuming the free rotation of CH_3 groups. In our ENDOR measurements, we could resolve six different hyperfine splittings for both the

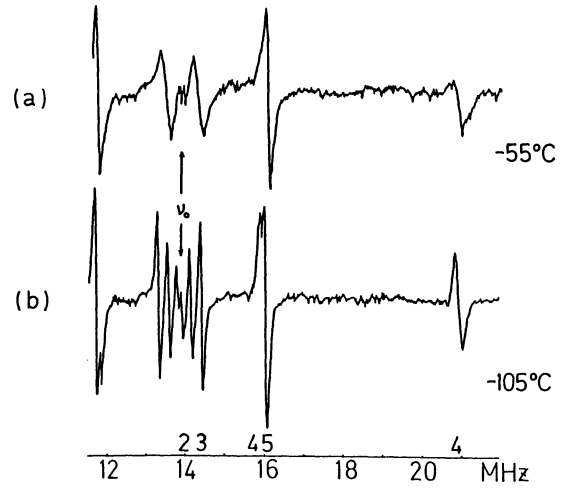


Fig. 4. ENDOR spectrum of cation radical **1** in CH_2Cl_2 at (a) −55 °C and (b) −105 °C.

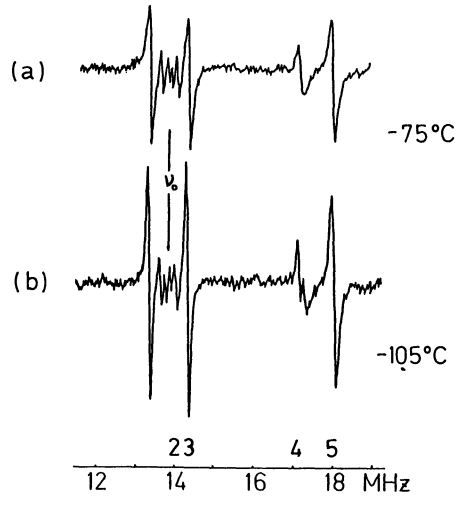


Fig. 5. ENDOR spectrum of cation radical **2** in CH_2Cl_2 at (a) −75 °C and (b) −105 °C.

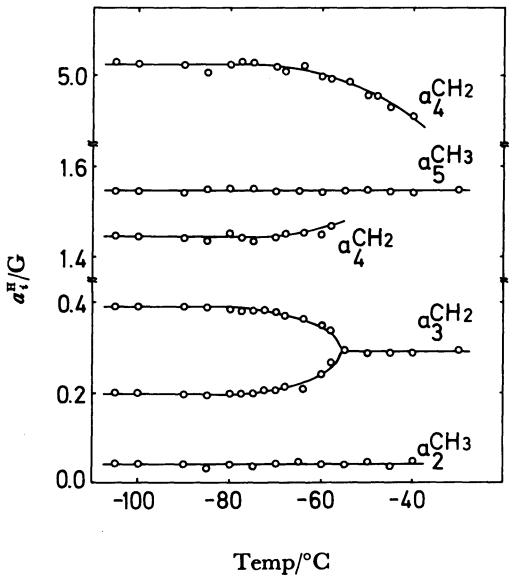


Fig. 6. Temperature dependence of the hyperfine couplings of cation radical **1** in CH_2Cl_2.

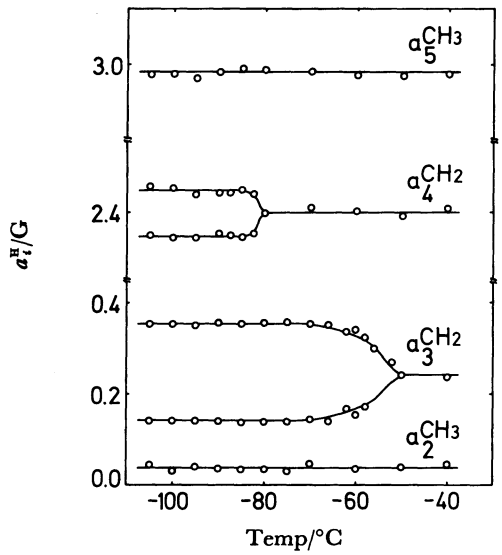


Fig. 7. Temperature dependence of the hyperfine couplings of cation radical **2** in CH_2Cl_2.

cation radicals **1** and **2**. Assignments of the hyperfine couplings have been performed, taking the temperature dependence of the hyperfine couplings (see Figs. 6 and 7) and the result of the McLachlan MO calculations (see Table 1), as described in the following section, into account.

McLachlan Molecular Orbital Calculations. McLachlan spin densities (ρ_i^π) of benzodipyran cation radicals were calculated, using the same parameters for the oxygen atom and methyl group ($\alpha_{O_1}=\alpha_{O_{1'}}=\alpha+2.0\beta$, $\beta_{C_{8a}-O_1}=\beta_{C_{8'a}-O_{1'}}=1.0\beta$, $\alpha_{C_{4a}}=\alpha_{C_{4'a}}=\alpha-0.06\beta$, $\alpha_{C_8}=\alpha_{C_{8'}}=\alpha-0.1\beta$, $\lambda=1.2$) as those used for the vitamin E radical (α-tocopheroxyl).[6] The results of McLachlan MO calculations (see Table 1) were found to be in satisfactory agreement with the "experimental" spin densities evaluated from the hyperfine coupling constants.

Conformation of β-Methylene Group in Benzodipyran Cation Radicals 1 and 2. The magnitude of the β-proton hyperfine coupling can often be calculated using Heller-McConnell's equation.[18]

$$a_\beta^H = \langle B_0 + B_2 \cos^2\theta \rangle \rho_i^\pi , \quad (1)$$

where ρ_i^π is the spin density on the carbon atom to which an alkyl group is attached and θ is the dihedral angle between the axis of the $2p_z$ orbital and the aliphatic C–H bond of the alkyl group. B_0 and B_2 are empirical parameters and were taken to be 0 and 54 G respectively, in Eq. 1. Consequently, the experimental values of the spin densities at C-5 and C-5′ carbon atoms were estimated from the methyl proton coupling, assuming the free rotation of the methyl group, $\langle\cos^2\theta\rangle=1/2$. On the other hand, in the ENDOR spectra for the cation radical **2** in CH_2Cl_2, two kinds of hyperfine couplings ($a_4^{CH_2}=2.452$ G and $a_4^{CH_2}=2.349$ G) (1 G=10^{-4} T) attributable to the magnetically inequivalent β-methylene protons were recorded at −105 °C. They collapsed into a single line as the temperature was raised to −80 °C (see Fig. 7). Therefore, in the case of the present cation radical **2**, one may expect that a rotation of the β-methylene residue would be tightly rocked at low temperatures. This is indeed true, because the temperature dependence of the β-proton coupling constant has been found to be negligible for the above cation radical **2** in the temperature range from −105 to −90 °C, as shown in Fig. 7. Consequently, the dihedral angles, θ_A and θ_B, of the inequivalent β-protons, were calculated to be 28.9 and 31.1° for **2**, respectively, assuming the sum of the two dihedral angles is $\pi/3$. The result suggests that the dihedral angle, θ_γ, between the C_3–C_4 bond and the aromatic ring is 1.1° (see Fig. 8). The experimental values of the spin densities at C-4a and C-4′a carbon atoms were estimated using Eq. 1.

Similarly, the dihedral angles (θ_A and θ_B) and the experimental spin densities of the cation radical **1** were calculated from the observed β-methylene hyperfine splittings (5.024 and 1.444 G) at −105 °C, assuming $\theta_A+\theta_B=\pi/3$. These values are listed in Tables 1 and 2.

As listed in Table 2, the values of dihedral angles (θ_A and θ_B) observed for **1** are very different from those observed for **2**. The above change in dihedral angles will be due to the difference in the steric repulsion forces between the β-methylene protons at C-4 and at C-4′ in **1** and between the β-methylene protons at C-4 and neighboring methyl protons

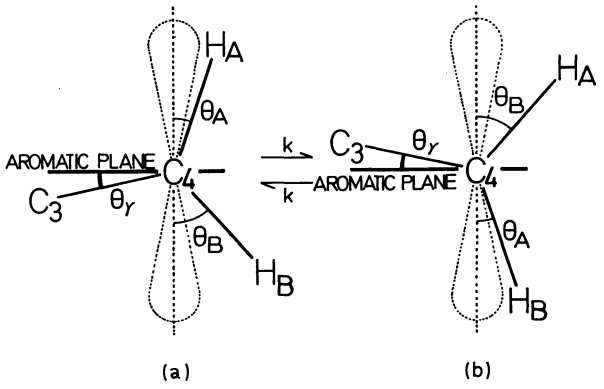


Fig. 8. Possible equilibrium conformations of the β-methylene group in cation radicals 1 and 2 in CH_2Cl_2.

TABLE 2. DIHEDRAL ANGLES (θ_A, θ_B AND θ_γ) AND ACTIVATION ENERGIES OF **1**, **2**, **3**, AND α-TOCOPHEROL MODEL

	θ_A/°	θ_B/°	θ_γ/°	E_{act} / kJ mol^{-1}	Solvent
1	2.4	57.6	27.6[b]	19.0±2.7	CH_2Cl_2
2	28.9	31.1	1.1[b]	32.8±4.6	CH_2Cl_2
3[a]	27.6	32.4	2.4[c]	25.9±1.7[c]	Toluene
α-Tocopherol model	—	—	11.1[d] 11.9	—	Solid state

a) 7-*t*-Butyl-2,2,5-trimethyl-6-chromanyloxyl (**3**). b) Calculated assuming $\theta_\gamma=|\theta_A-\theta_B|/2$. c) From Ref. 19. d) There are two different molecules in the unit cell. From Ref. 13.

at C-5′ in **2**.

As described in a previous section, Burton and Ingold have reported that the dihedral angle, θ_γ, between the aromatic ring and the C_3–C_4 bond is 11.1 and 11.9°, from an X-ray crystallographic analysis of α-tocopherol model.[13] In the α-tocopherol model and the above cation radical **2**, the steric repulsion force between the β-methylene protons at C-4 and the neighboring methyl protons (at C-5 in α-tocopherol model and at C-5′ in **2**) will be similar to each other, and thus the conformation of the heterocyclic ring in **2** is considered to be similar to that of the α-tocopherol model. However, the value of the dihedral angle (θ_γ=1.1°) in **2** is different from those (θ_γ=11.1 and 11.9°) in α-tocopherol model. On the other hand, very recently, we have succeeded in measuring the ENDOR spectrum of a new stable tocopheroxyl radical **3** (7-*t*-butyl-2,2,5-trimethyl-6-chromanyloxyl) (see Table 2) in toluene.[19] It was found that the two β-methylene protons in the heterocyclic ring become magnetically inequivalent at low temperature (<−72 °C). The observed dihedral angles are θ_A=27.6° and θ_B=32.4°, and θ_γ is calculated to be 2.4°. These values are very similar to those of the cation radical **2**, because the steric repulsion force between the β-methylene protons at C-4 and the neighboring methyl protons is equivalent in **2** and **3**. These results suggest that the solution structure of the heterocyclic ring attached to the aromatic ring is similar to each other and the crystal and solution conformations of the heterocyclic ring are distinctly different.

Dynamic Behavior of Heterocyclic Ring. As described in a previous section, in the ENDOR spectra of the cation radical **2** in CH_2Cl_2, two signals attributable to the two inequivalent splittings of the β-methylene protons ($a_4^{CH_2}$=2.452 G and $a_4^{CH_2}$=2.349 G) were recorded at −105 °C. The β-methylene proton hyperfine coupling constants remain almost unchanged upon warming from −105 to −85 °C. They collapsed into a single line ($a_4^{CH_2}$=2.401 G) as the temperature was raised to −80 °C (see Fig. 7). Similar behavior has been observed for γ-methylene protons.

In such a case, we can calculate the activation energy E_{act} for the conformational interconversion of the heterocyclic ring, applying the Gutowsky-Holm equation.[20–22] In a four-jump dynamic process, the separation $\Delta\omega$ on the two ENDOR lines at T K due to β- or γ-methylene protons is given by

$$\Delta\omega = (\Delta\omega_{max}^2 - 8k^2)^{1/2} \quad (2)$$

where $\Delta\omega_{max}$ is the two limiting values of the splitting constants of β- or γ-methylene protons and equals to $\gamma_e|(a_A^{CH_2}-a_B^{CH_2})/2|$, and $k=1/2\tau$. Here, τ is the mean life time of each conformation.

With the aid of this relation we have obtained k at different temperatures for cation radical **2** in CH_2Cl_2. However, in the case of the β-methylene protons, the value of $\Delta\omega_{max}$(=0.103 G=0.289 MHz) is very small and thus the change of the $a_4^{CH_2}$ values occurs in the narrow temperature range (−85—−80 °C). Therefore, the E_{act} value was obtained from the temperature dependence of γ-methylene proton hyperfine couplings ($a_3^{CH_2}$). Figure 9 shows the Arrhenius plots of $\log k$ *vs.* $1/T$ for **2** from which E_{act} was determined. The observed activation energy E_{act}=32.8±4.6 kJ mol^{-1}. On the other hand, in the case of cation radical **1**, the value of $\Delta\omega_{max}$(=3.580 G=10.033 MHz) for β-methylene hyperfine coupling is very large and the coalescence temperature is higher than the temperature where the ENDOR observation is possible. Therefore, the E_{act} was calculated from the temperature dependence of γ-methylene proton hyperfine couplings, as performed for cation radical **2**. The activation energy obtained is E_{act}=19.0±2.7 kJ mol^{-1}. As described in a previous section, in the cation radical **2** and the tocopheroxyl radical **3** (7-*t*-butyl-2,2,5-trimethyl-6-chromanyloxyl), the steric repulsion force between the β-methylene protons at C-4 and the neighboring methyl protons will be similar to each other, and thus we can expect similar E_{act} values for **2** and **3**. However, the activation energy, E_{act} obtained in the present work for **2** is slightly larger than that (25.9±1.7 kJ mol^{-1}) reported for such an interconversion in **3**.[19]

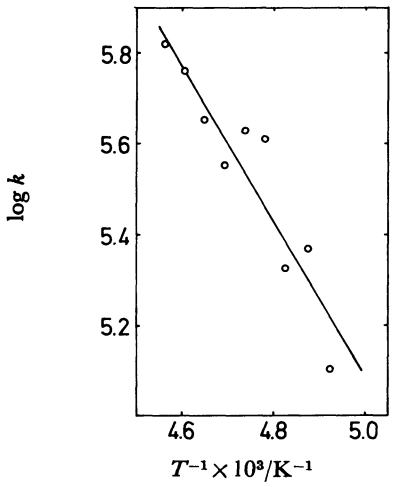


Fig. 9. The Arrhenius plot of the rate constant for the conformational interconversion process of the heterocyclic ring of cation radical **2** in CH_2Cl_2.

References

1) P. Schudel, H. Mayer, and O. Isler, "The Vitamins," 2nd ed, ed by W. H. Sebrell, Jr. and R. S. Harris, Academic Press, New York (1972), Vol 5, pp. 168—218.

2) M. L. Scott, "The Fat-Soluble Vitamins," ed by H. F.

DeLuca, Plenum Press, New York (1978) pp 133—210.
3) D. H. Kohl, J. R. Wright, and M. Weissman, *Biochim. Biophys. Acta*, **180**, 536 (1969).
4) W. Boguth and H. Niemann, *Biochim. Biophys. Acta*, **248**, 121 (1971).
5) T. Ozawa, A. Hanaki, S. Matsumoto, and M. Matsuo, *Biochim. Biophys. Acta*, **531**, 72 (1978).
6) K. Mukai, N. Tsuzuki, K. Ishizu, S. Ouchi, and K. Fukuzawa, *Chem. Phys. Lipids*, **29**, 129 (1981).
7) K. Mukai, N. Tsuzuki, S. Ouchi, and K. Fukuzawa, *Chem. Phys. Lipids*, **30**, 337 (1982).
8) J. Tsuchiya, E. Niki, and Y. Kamiya, *Bull. Chem. Soc. Jpn.*, **56**, 229 (1983).
9) M. Matsuo and S. Matsumoto, *Lipids*, **18**, 81 (1983).
10) K. Mukai, K. Takamatsu, and K. Ishizu, *Bull. Chem. Soc. Jpn.*, **57**, 3507 (1984).
11) G. W. Burton, Y. Le Page, E. J. Gabe, and K. U. Ingold, *J. Am. Chem. Soc.*, **102**, 7792 (1980).
12) G. W. Burton and K. U. Ingold, *J. Am. Chem. Soc.*, **103**, 6472 (1981).
13) T. Doba, G. W. Burton, and K. U. Ingold, *J. Am. Chem. Soc.*, **105**, 6505 (1983).
14) G. W. Burton, L. Hughes, and K. U. Ingold, *J. Am. Chem. Soc.*, **105**, 5950 (1983).
15) J. L. G. Nilsson, H. Sievertsson, and H. Selander, *Acta Pharm. Suecica*, **5**, 215 (1968).
16) J. L. G. Nilsson, H. Sievertsson, and H. Selander, *Acta Chem. Scand.*, **22**, 3160 (1968).
17) J. J. Windle and A. K. Wiersma, *J. Chem. Phys.*, **39**, 1139 (1963).
18) C. Heller and H. M. McConnell, *J. Chem. Phys.*, **32**, 1535 (1960).
19) K. Mukai, K. Takamatsu, and K. Ishizu, sent for publication.
20) H. S. Gutowsky and C. H. Holm, *J. Chem. Phys.*, **25**, 1228 (1956).
21) C. Morczyskowski, K. Möbius, and M. Plato, *J. Magn. Reson.*, **17**, 202 (1975).
22) M. Iwaizumi, S. Kita, M. Kohno, and T. Isobe, *Bull. Chem. Soc. Jpn.*, **53**, 1745 (1980).