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Carboxylation of the Nitroxide Radical of 2,2,6,6-Tetramethyl-4-piperidone-1-oxyl with Carbon Dioxide and Potassium Phenoxide, and the Physical Properties of the Products

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A stable nitroxide radical containing active methylenes, 2,2,6,6-tetramethyl-4-piperidone-1-oxyl (I), was found to be carboxylated by potassium phenoxide and carbon dioxide in an aprotic solvent (N,N-dimethylformamide), producing new nitroxide compounds. The mono- or dicarboxylate was formed selectively, depending on the molar ratio of the substrate to potassium phenoxide.

The ultraviolet and visible spectra of the monomethyl- (II) and dimethylester (III) of the carboxylates as well as those of I and 2,2,6,6-tetramethylpiperidine-1-oxyl (IV) were investigated in various solvents. The results indicated that II exists mainly as the keto form in a polar solvent, and as the enol form in a nonpolar solvent, while the enol form of III is predominant in all the solvents tested. The nitrogen hyperfine coupling constants, A_N, of I—IV in the electron spin resonance spectra were determined, and the results are discussed in terms of the keto-enol equilibrium.

Furthermore, the distribution coefficients of these nitroxide radicals between hexane and water were measured to assess the potential suitability of these newly formed nitroxide radicals for use as spin labels or probes.

Keywords——carboxylation; nitroxide radical; spin label; electronic spectra; ESR; solvent effect; keto-enol

We have already shown that the carboxylation of organic compounds that contain active methylenes, such as cyclohexanone, can be achieved under very mild conditions with potassium phenoxide and carbon dioxide in N,N-dimethylformamide (DMF).[2] In the present paper we wish to report that the carboxylation of a nitroxide radical is also possible under the same conditions to yield another nitroxide radical. The reaction substrate, 2,2,6,6-tetramethyl-4-piperidone-1-oxyl (I), contains a carbonyl group and is employed as a starting material in syntheses of its derivatives,[3] which are generally utilized as spin labels or spin probes for biopolymers or synthetic polymers.[4] Thus, we also investigated the electronic spectra and the electron spin resonance (ESR) spectra of the reaction products, in order to assess their suitability for use as spin labeles or spin probes.

Experimental

(1) **Synthesis of I**——The substrate, I, was synthesized[5] by the oxidation of triacetoamine with hydrogen peroxide in the presence of sodium tungstate as a catalyst. mp 36.5—37° (lit,[5] mp 36.5°) *Anal.* Calcd for $C_9H_{16}NO_2$: C, 63.50; H, 9.47; N, 8.24. Found: C, 63.11; H, 9.64; N, 8.10. Yield 49%.

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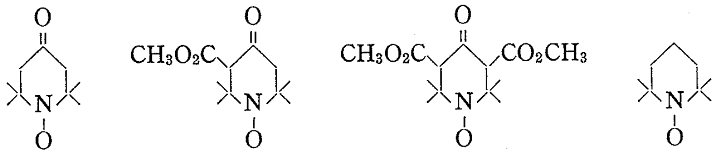
(2) **The Carboxylation of I with Potassium Phenoxide at a Molar Ratio of 1 : 2**——Carbon dioxide was bubbled into a solution of 2.64 g (0.02 mol) of potassium phenoxide in 80 ml of DMF at room temperature for 20 min, then 1.70 g (0.01 mol) of I was added to the solution. After two hours, the mixture was acidified with dilute hydrochloric acid, and extracted with diethyl ether. Carboxylated products were extracted from the ethereal extract with 10% aqueous sodium carbonate, while phenol remained in the ethereal layer. The aqueous solution of carboxylates was then acidified and extracted with diethyl ether. An ethereal solution of diazomethane was added to this extract in order to methylate the carboxylic acid. The ethereal solution was dried over anhydrous sodium sulfate, and removal of the solvent by evaporation yielded orange crystals, which were recrystallized from petroleum ether. mp 70—71.5°. *Anal.* Calcd for $C_{11}H_{18}NO_4$: C, 57.88; H, 7.95; N, 6.14. Found: C, 57.74; H, 8.08; N, 6.04. IR ν_{max}^{KBr} cm^{-1}: 3390 (OH), 1648 (C=O), 1608 (C=C). MS *m/e*: 228 (M^+). The ESR spectrum of the product in benzene (1×10^{-4} M) gave three lines due to the interaction of the unpaired electron with the ^{14}N nucleus. In view of these data the product was assigned as 2,2,6,6-tetramethyl-3-methoxycarbonyl-4-piperidone-1-oxyl (II). Judging from the infrared (IR) spectrum, we concluded that the enol form was predominant in the solid state. Yield 35%.

(3) **The Carboxylation of I with Potassium Phenoxide at a Molar Ratio of 1 : 20**——The carboxylation was carried out with a large excess of potassium phenoxide, and the reaction mixture was treated in the manner described above. Orange crystals were obtained. mp 113—114°. *Anal.* Calcd for $C_{13}H_{20}NO_6$: C, 54.54; H, 7.04; N, 4.89. Found: C, 54.67; H, 7.10; N, 4.96. IR ν_{max}^{KBr} cm^{-1}: 3430 (OH), 1742 (C=O), 1660 (C=O), 1615 (C=C). MS *m/e*: 286 (M^+). The ESR spectrum of this compound in benzene (1×10^{-4} M) gave three lines. The IR spectrum indicated that this radical existed largely as the enol form in the solid state. In view of these data, this compound was assigned as 2,2,6,6-tetramethyl-3,5-bis(methoxycarbonyl)-4-piperidone-1-oxyl (III). Yield 39%.

(4) **Synthesis of 2,2,6,6-Tetramethylpiperidine-1-oxyl (IV)**——The compound IV was synthesized as follows, in order to compare the physical properties of II and III with those of IV. Triacetoamine was reduced with hydrazine[6] to form 2,2,6,6-tetramethylpiperidine. This compound was oxidized with hydrogen peroxide in the presence of sodium tungstate as a catalyst to yield IV.

(5) **Spectral Measurements**——Ultraviolet (UV) and visible spectra were measured with a Hitachi EPS-3T spectrometer, IR spectra with a EPI-G3 machine and ESR spectra with a JEOL PE-1X machine. The ESR samples were degassed and the concentration of the radical solution was about 1×10^{-4} M.



I II III IV

Chart 1

Results and Discussion

As already mentioned, a nitroxide radical can be carboxylated to yield new radical products; specifically, the new radicals II and III were prepared by carboxylation of the substrate I. The physical properties of II and III were studied and compared with those of the known compounds I and IV.

(1) UV and Visible Spectra

Nitroxide radicals have absorption maxima both in the UV region (230—250 nm) and in the visible region (410—460 nm). The former is ascribable to the π-π^* transition of the nitroxide group, and has a large molar absorption coefficient (ε value). The latter, ascribable to the n-π^* transition, has a small ε value. The absorption maxima and ε values were measured in various solvents at room temperature, and the results are shown in Table I. The changes in the spectra of II and III were followed for more than a week after dissolving the compounds, and ε values were determined when they reached constant values. Those of II became constant after one day in water, and after seven days in acetonitrile. The absorption spectra of II, III,

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[6] R. Brière, H. Lemaire, and A. Rassat, *Bull. Soc. Chim. Fr.*, 32, 3273 (1965).

Table I. UV and Visible Absorption Data for Nitroxide Radicals in Various Solvents

Compound	Hexane		Ethanol		Acetonitrile		Water	
	λ_{max}[a]	ε[b]	λ_{max}	ε	λ_{max}	ε	λ_{max}	ε
I	236	2380	236	2270	236	2190	237	2500
	457	6.8	436	6.6	442	7.9	413	7.5
II	252	10000	251	9220	250	7160	247	3780
	455	8.6	440	10.1	452	9.1		—
III	253	9360	251	9940	249.5	9800	249	9550
	451	9.9	440	9.9	445	9.9		—
IV	243	1660	244	2160	244	1710	246	2070
	470	10.5	452	13.0	462	11.0		—

a) The absorption maximum (λ_{max}) is given in nm.
b) The molar absorption coefficient (ε) is given in $\text{M}^{-1}\cdot\text{cm}^{-1}$.

and IV in the visible region could not be measured in water due to the low solubility of the compounds and the small ε values.

The absorption maxima of II and III in the UV region were red-shifted from that of I by 10—17 nm in all solvents. The ε values were also increased. II gave a value of about 10000 in nonpolar solvents such as hexane, but a comparatively small value of about 4000 in polar solvents such as water. In contrast, III gave a value of 9000—10000 in all the solvents investigated. Since the contribution of a nitroxide group to the ε value is about 2000, as shown by the values of I and IV, the remainder must be due to other groups. In the case of β-ketoesters such as acetoacetate[7] and ethyl cyclohexane-2-one carboxylate,[8] it is known that the keto-enol equilibrium depends on the polarity of the solvent. That is, in a nonpolar solvent, enolization of the β-ketoesters occurs, accompanied by an increase of the ε values. For example, the ε value of ethyl cyclohexane-2-one carboxylate at the absorption maximum (258 nm) is 6420 in ethanol, and 10200 in cyclohexane (in which the enol form is predominant). Similarly, the finding that II and III afford large ε values in nonpolar solvents may also be ascribed to the predominance of the enol form. This consideration is supported by the fact that no keto form was apparent in the IR spectra of II and III in carbon tetrachloride.

Thus, in aqueous solution, III showed as large an ε value as in hexane, while II gave a value less than half as large. This suggests that III may retain the enol form in water, while a large fraction of II may be transformed into the keto form in the same solvent. In fact, the IR spectrum of II measured in a polar solvent such as acetonitrile or dimethyl sulfoxide (DMSO) showed absorption due to the keto form. The IR spectrum of II in acetonitrile, presented in Fig. 1, exhibited absorptions at 1745 cm^{-1} (C=O of ester in the keto form) and 1720 cm^{-1} (C=O of ketone in the keto form). The keto form was not observed in the IR spectrum of III in DMSO.

Thus, it may be concluded that III tends to exist predominantly in the keto form, in contrast to II. This may be interpreted by assuming marked stabilization of the enol form of III due to the introduction of the two methoxycarbonyl groups, which can interact with the enol proton to form a six-membered chelate. The apparent red-shift of the absorption maxima of II and III compared with that of I in the UV region can be ascribed to overlapping of the absorption due to the enol form, which possesses a large ε value, and absorption maxima at longer wavelength than that of the normal nitroxide group. The λ_{max} of the enol form may be at about 258 nm in view of the data on ethyl cyclohexane-2-one carboxylate.

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7) A.S.N. Murthy, A. Balasubramanian, C.N. Rao, and T.R. Kasturi, *Can. J. Chem.*, **40**, 2267 (1962).
8) S.J. Rhoads, J.C. Gilbert, A.W. Decora, T.R. Garland, R.J. Spangler, and M.J. Urbigkit, *Tetrahedron*, **19**, 1625 (1963).

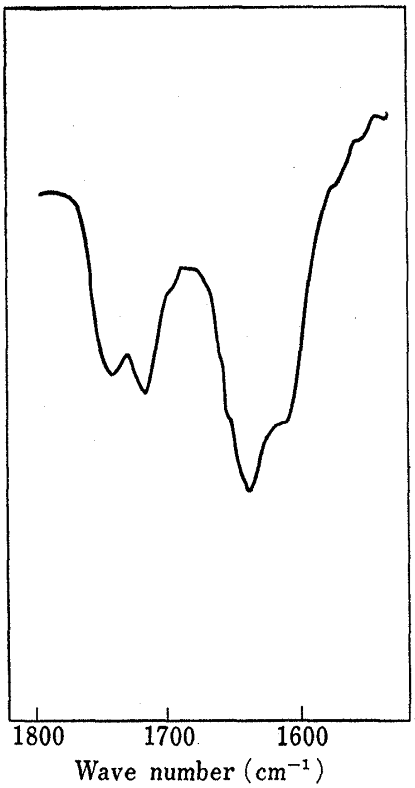

Fig. 1. IR spectrum of II in Acetonitrile

Table II. *g*-Values for Nitroxide Radicals in Benzene and Water

Compound	Benzene	Water
I	2.0062	2.0057
II	2.0061	2.0057
III	2.0061	2.0057

(2) **ESR Spectra**

Nitroxide radicals in solution afford three lines in the ESR spectra as a result of the hyperfine interaction between the unpaired electron and the nitrogen nucleus (^{14}N). Table II lists the g-values observed in benzene and water. The nitroxide I—III gave similar g-values in the same solvent. The nitrogen hyperfine coupling constants, A_N, were also determined in various solvents as shown in Table III. The results indicate that in the case of I, III, and IV, the A_N value increases in the following order: benzene$<$acetonitrile $\approx$DMSO$<$ethanol$<$water. The A_N values of nitroxide radicals are generally increased in solvents where the mesomeric structure (B) (see chart 2) is favored. It is expected that the polarized form, (B), will be favored in solvents having a high dielectric constant (ε), leading to an increased A_N value. However, the A_N value in ethanol is greater than that in DMSO or acetonitrile despite the lower ε value. It is known that IV forms an adduct with alcohol or phenol by hydrogen honding.[9] If the hydrogen bond is formed with lone pair electrons of oxygen in the nitroxide radical, the contribution of the structure (B) may increase, with a corresponding increment of the A_N value. It is noteworthy that solvents which increase A_N do not always favor the keto form.

Table III. Nitrogen Hyperfine Coupling Constants (A_N) for Nitroxide Radicals in Various Solvents (in gauss)

Compound	Benzene (2.28)[a]	Acetonitrile (37.5)[a]	DMSO (46.68)[a]	Ethanol (24.55)[a]	Water (78.39)[a]
I	14.5	14.8	14.8	15.0	16.0
II	15.3	15.2	15.4	15.8	16.1
III	15.4	15.6	15.7	15.8	16.6
IV	15.5	15.9	15.9	16.3	17.2

a) Dielectric constants are cited from ref. 10.

The difference between the A_N value of II in water and that in ethanol was found to be 0.3 G, in contrast to the difference of about 1.0 G in the case of other nitroxide radicals. It is known for cyclic six-membered nitroxide radicals with two *gem*-dimethyl groups at the 2- and 6-positions that those with a polar functional group at the 4-position show smaller A_N values than those with a nonpolar functional group at this position.[6] This effect is illustrated by

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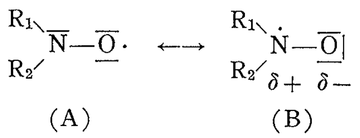

$$\begin{matrix}R_1 \\ & N-\bar{\underline{O}}\cdot \\ R_2\end{matrix} \longleftrightarrow \begin{matrix}R_1 \\ & \dot{N}-\overline{\underline{O}}| \\ R_2 & \delta+ \quad \delta-\end{matrix}$$

Chart 2

the observation that the A_N value of I (16.0 G in water) is smaller than that of IV (17.2 G in water). On the basis of these results, it may also be assumed that the keto form of II has a smaller A_N value than the enol form. Thus, it may be supposed that the transformation of II from the enol form (in ethanol) to the keto form (in water) reduced the solvent effect upon the A_N value. Analogously, the observation that the A_N value of II in acetonitrile or DMSO is comparable with that in benzene, in constrast to the cases of the other compounds, may be interpreted in terms of the existence of a considerable amount of the keto form in this solvent. Actually, in the ESR spectrum of II in acetonitrile (Fig. 2), the central peak height was rather different from the lower field peak height (1 : 0.60), and the linewidth of the central absorption line (ΔH) was 0.9 G, while that of the lower field line was 1.4 G. These results indicate overlapping of the absorption lines attributed to the keto form and the enol form.

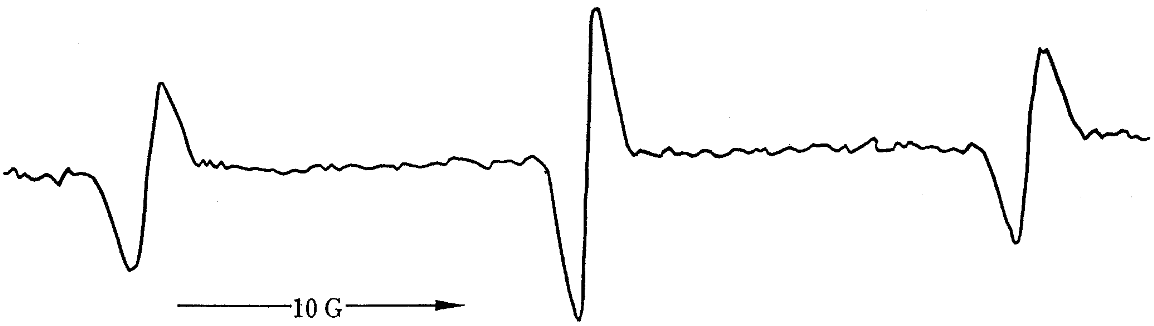

Fig. 2. ESR Spectrum of II in Acetonitrile

The difference between the A_N value of II and that of III in ethanol or benzene was only 0.1 G, indicating that the presence of a methoxycarbonyl group at the 5-position has very little effect upon the A_N value. It also appears that the methoxycarbonyl group at the 3- position makes little contribution to A_N. Thus, the A_N value of I and II in water, where II exists predominantly as the keto form, are almost the same.

(3) Equilibrium Distribution of the Reaction Products between Hexane and Water

In order to investigate the hydrophobic (or hydrophilic) character of the reaction products, their distribution between hexane and water was observed at room temperature (30°) by UV titration. About 1×10^{-5} mol of nitroxide radical was dissolved in a mixture of equal volumes (50 ml) of hexane and water. Equilibrium was established by stirring for one hour. The distribution coefficients were determined at three or four concentrations, and the average coefficients for the nitroxide radicals, I—IV, are given in Table IV. As shown in Table IV, the distribution coefficients were found to be markedly increased by the introduction of a methoxycarbonyl group. It is noteworthy that the hydrophobic character of III is almost the same as that of IV.

Table IV. Distribution Coefficients of Nitroxide Radicals between Hexane and Water

Compound	Distribution coefficient
I	0.30±0.01
II	20±1
III	31±1
IV	37±1

The compound IV has been utilized as a spin label for the study of biomembranes.[11] The radical IV seems to be distributed in hydrophobic regions as a result of its hydrophobic character. The nitroxide radicals, II and III, have not yet been used as spin labels. In view of their physical and chemical character, however, they should be useful as spin labels or spin probes. Moreover, they may be used as ligands in metal complexes, because complex formation between an enolizable β-ketoester and ferric chloride is a well-known reaction.

Conclusion

(1) The nitroxide radical, I, can be carboxylated with potassium phenoxide and carbon dioxide to form a mono- or dicarboxylate without losing its radical character.

(2) Of the reaction products, the nitroxide radical III exists as the enol form in all solvents investigated, while the radical II coexists as the keto form and the enol form. The proportion of the two forms depends upon the polarity of the solvent. The absorption coefficients and the nitrogen hyperfine coupling constants of II and III in various solvents can be reasonably interpreted in terms of keto-enol ratio shifts.

(3) The nitroxide radicals, II and III, are quite hydrophobic and may be useful as spin probes for studies of biomembranes.

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