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162. Nobuko Kataoka, Akira Imamura, Yutaka Kawazoe, Goro Chihara, and Chikayoshi Nagata : Electron Spin Resonance Study on the Photoinduced Radicals from Related Compounds to Carcinogenic 4-Nitroquinoline 1-Oxide*[1]

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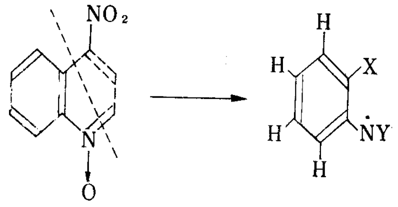
In our previous paper[1)] it was reported that strongly carcinogenic 4-nitroquinoline 1-oxide (4-NQO) was found by electron spin resonance (ESR) technique to produce several kinds of stable free radicals when it was irradiated by ultra violet or visible light in solution. The radical production was strongly dependent on the solvent used, three different radicals being identified on ESR spectra obtained from its dioxane, benzene, and hexane solutions, respectively. Their chemical structures were discussed by analyzing hyperfine structures of their ESR spectra with a help of the isotope (^{15}N or D)-containing 4-NQOs. The radical structure produced in dioxane could be determined as structure A (in Chart 1), the unpaired electron being delocalized over the ring nitrogen and the benzene moiety of this molecule but not or scarecely over the pyridine moiety nor the nitro group nitrogen. The radical produced in the benzene solution could be characterized as the one which had the unpaired electron localized mainly on the nitrogen of the substituent nitro group. The third radical which was different from either of the above ones was produced in the hexane solution, the unpaired electron of which was also localized mainly on the substituent nitro group nitrogen.



(A)

Chart 1.

These findings prompted a further investigation of this photodegradation reaction in more detail using a variety of quinoline derivatives, intending in mind to obtain informations of the role of 4-NQO derivatives playing in the carcinogenicity on the skin of animals. This paper describes the ESR spectral features of about twenty kinds of the quinoline derivatives photoirradiated in dioxane and benzene solutions. The relation between the photochemical behavior and the chemical structures will be discussed.

Experimental

Compound——The compounds used were synthesized and purified by the authentic preparative methods. They are listed in Table II, classified to groups A, B, and C according to their photochemical behaviors which will be described in a following section. The solutions for irradiation were prepared so as to contain 10~20 mg. of the compound examined in 1 ml. of photo-graded dioxane or benzene. 4-Hydroxyaminoquinoline 1-oxide (4-HAQO) solution contained only several γ of the solute because of its extremely poor solubility.

UV-irradiation——1 ml. of the solution examined was irradiated for 10~20 minutes in an ESR sample tube of quarts (0.5 cm. in diameter) by a 100 Watt high pressure mercury lamp (Ushio Kogyo's UM 100 type) from a distance of 6 cm. under running-water cooling. In cases where they did not give any ESR signals

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*[1] This paper constitutes Part III of a series entitled "ESR Studies on 4-Nitroquinoline 1-Oxide and Its Related Compounds." This work has been reported at the 21th Annual Meeting of Pharmaceutical Society of Japan.

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1) N. Kataoka, A. Imamura, Y. Kawazoe, G. Chihara, C. Nagata : This Bulletin, **14**, 897 (1966).

under the above condition, UV-irradiation was carried out during ESR measurement at a distance of 30 cm. using the same lamp which was attached to our ESR spectrometer.

ESR Spectra——All spectra were measured at room temperatures soon after or during UV-irradiation by a Japan Electron Optics Laboratory's JES-3BX spectrometer with 100 kc./sec. field modulation.

Results

Photodegradation of Substituted 4-Nitroquinoline 1-Oxides All 4-NQO derivatives examined (listed in group A of Table II), except for 3-methyl-4-NQO and 4,8-dinitroquinoline 1-oxide, produced two kinds of free radicals whose structures were proved to be the same types as those produced by UV-irradiation of the dioxane, and benzene solutions of 4-NQO, respectively. Thus, one is a radical whose unpaired electron was delocalized over the benzene moiety and the ring nitrogen (structure A), whereas the other has an unpaired electron localized mostly on the nitro group nitrogen. The former will be called as type I and the latter as type II in this paper.

The free radical structure could be easily characterized by the analyses of hyperfine structures of the ESR spectra with a help of the isotope containing derivatives, I, II, III, and IV in Chart II.

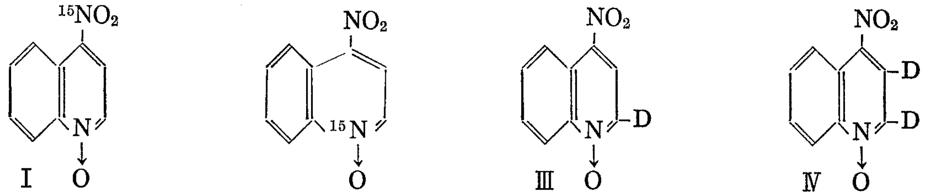

Chart II.

Thus, replacement of ^{14}N by ^{15}N results in a significant change in signal pattern (from triplet to doublet) and in magnitude of the coupling constant A ($A_{^{15}N}=1.403\ A_{^{14}N}$). Replacement of H by D also causes spectral changes, that is, from doublet to triplet and from A_H to 0.154 A_H[*3]. Although the radical formation was dependent on the sort of the solvent used, it was not always to produce only one kind of radical in each solvent. Some compounds produced both radicals of the type I and II in dioxane or benzene simultaneously, and some produced no radical in the benzene solution. The spectra of the compounds examined are reproduced in Fig. 1 including schematic

TABLE I. Magnetic Resonance Parameters

	Type I radical				Type II radical			
					in Dioxane		in Benzene	
	g-Value	A_N	$A_{H_{o,p}}$	A_{H_m}	g-Value	A_N	g-Value	A_N
4-NQO	2.005_0	6.2	2.3	0.7	2.004_0	27.4	2.004_0	27.3
2-Methyl-4-NQO	2.004_9	9.2	3.1	0.9			2.003_7	27.4
6-Methyl-4-NQO	2.005_4	5.8	2.9	—a)	2.003_5	29.7		
7-Methyl-4-NQO	2.005_1	6.0	3.0	—a)	2.004_0	27.6	2.004_1	27.4
6-Chloro-4-NQO	2.005_0	10.0	3.1	—a)	2.004_4	28.0		
7-Chloro-4-NQO	2.004_7	9.3	2.9	—a)	2.003_8	28.4	2.004_0	28.5

a) The splittings due to meta-hydrogen could not be detected.

[*3] Spin numbers of H, D, ^{14}N, and ^{15}N are 1/2, 1, 1, and 1/2, respectively. Magnetogyric ratios of these isotopes are as follows: $\gamma_D/\gamma_H=0.154$, $\gamma_{^{15}N}/\gamma_{^{14}N}=1.403$.

TABLE II. Compounds

The related compounds to 4-NQO examined	m.p. (C°)	References	
		Synthesis	Carcinogenesis
Group A			
4-nitroquinoline 1-oxide	153~154	(2)	(20)
2-methyl-4-NQO	157	(3)	(21)
6-methyl-4-NQO	184~186	(4)	
7-methyl-4-NQO	164~165		
6-chloro-4-NQO	193~195	(4)	(21)(22)
7-chloro-4-NQO	219	(5)	(22)
Group B			
3-methyl-4-NQO	179~180	(6)	
5-nitroquinoline 1-oxide	161	(2)(7)	
Group C			
4-nitroquinoline	87	(8)(9)	(21)
3-nitroquinoline 1-oxide	191~192	(10)(11)	
6-nitroquinoline 1-oxide	221	(12)	
7-nitroquinoline 1-oxide	174~175	(11)	
8-nitroquinoline 1-oxide	180~181	(2)(7)	
4,8-dinitroquinoline 1-oxide	222~223 (decomp.)	(7)	
4-nitropyridine 1-oxide	159	(13)	
4-hydroxyaminoquinoline 1-oxide	220~224 (HCl salt 193~196)	(14)(15)(16)	(23)(24)(25)
4-aminoquinoline 1-oxide	272 (decomp.)	(17)	(26)
4-chloroquinoline 1-oxide	133~133.5	(18)	
quinoline 1-oxide	60~62 (hydrate	(19)	(21)
quinoline			

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illustration of the spectra analyses. Some examples of the spectral changes by isotope replacement are shown in Fig. 2. The magnetic resonance parameters are summarized in Table I. All the g-values of the Type I radicals are about 2.005 and those of the Type II are about 2.004 independently of the sort of the solvents and the substituents on the aromatic skeleton. The g-value of 3-methyl-4-NQO is 2.005_3 in dioxane and 2.005_6 in benzene, and that of 5-NQO is 2.005_3 in dioxane and 2.005_2 in benzene.

It is of interest to note that the presence of oxygen was required for production of type II radical. Thus, degassing at an extent of 10^{-4} mm. Hg was enough for inhibition of Type II radical formations in every case of group A.

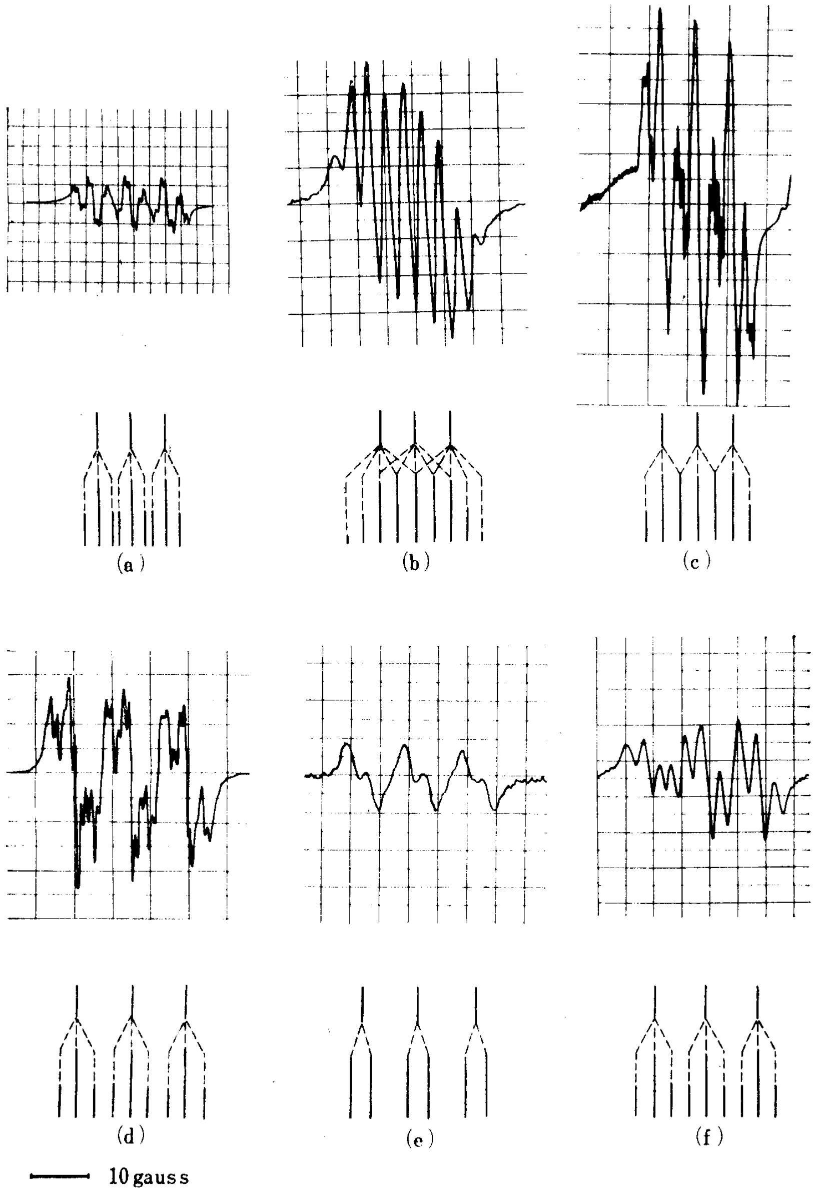


Fig. 1. Hyperfine Structures of the Type I Radicals obtained from 4-NQO and Its Derivatives and Their Analyses

The splittings due to ring nitrogen and o,p-hydrogens are presented.
(a) 4-NQO (b) 6-methyl-4-NQO (c) 7-methyl-4-NQO
(d) 2-methyl-4-NQO (e) 6-chloro-4-NQO (f) 7-chloro-4-NQO

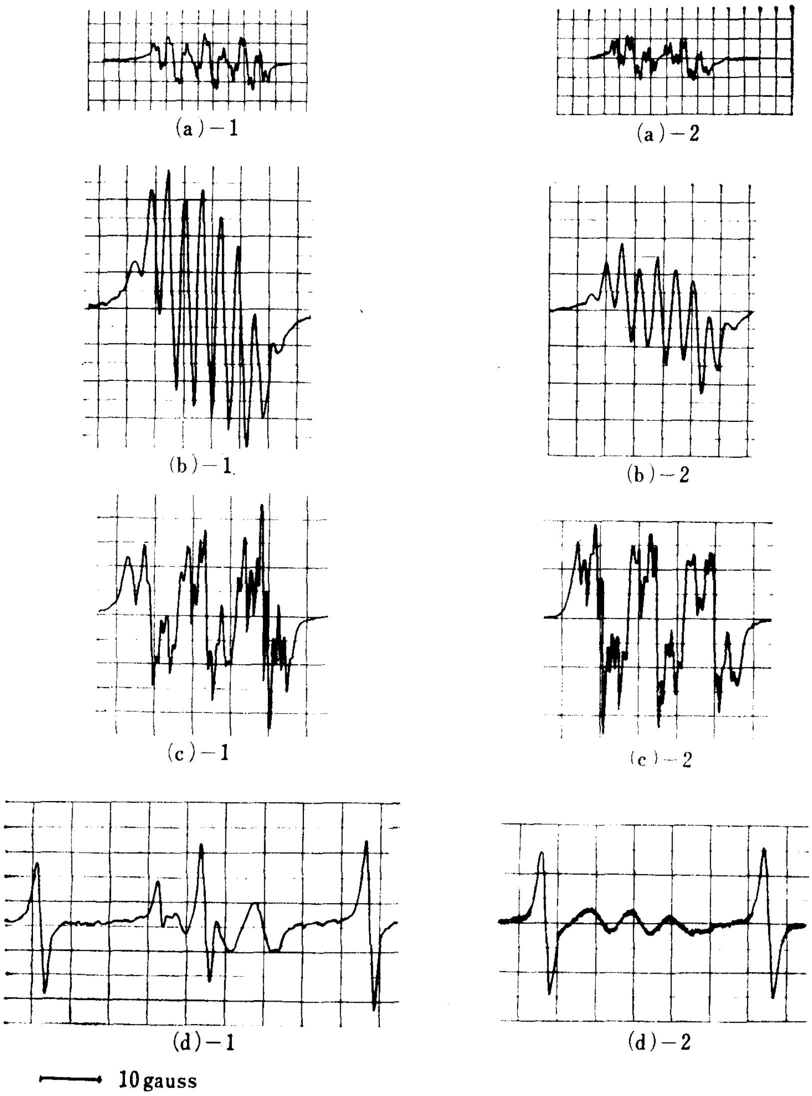
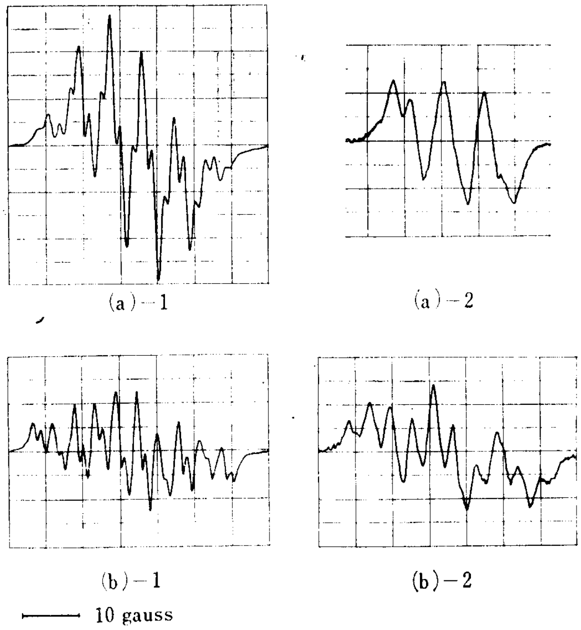


Fig. 2. Spectral Changes due to Isotope Replacement

(a) ^{15}N-replacement of the ring nitrogen (in dioxane)
(a)-1: 4-NQO (a)-2: ring-^{15}N-4-NQO
(b) ^{15}N-replacement of the nitro group nitrogen (in dioxane)
(b)-1: 6-methyl-4-NQO (b)-2: $^{15}NO_2$-6-methyl-4-NQO
(c) D-replacement of the hydrogen at 2-position of the quinoline ring (in dioxane)
(c)-1: 2-methyl-4-NQO (c)-2: 2-deutero-2-methyl-4-NQO
(d) ^{15}N-replacement of the nitro group nitrogen (in benzene)
(d)-1: 7-methyl-4-NQO (d)-2: $^{15}NO_2$-7-methyl-4-NQO

2-Methyl-4-NQO: Type I radical (Fig. 1, d) was obtained in dioxane and in benzene. ^{15}N-Replacement of nitro group nitrogen did not affect signal pattern and D-replacement of hydrogen-2 (Fig. 2, c) and -3 did not affect either. The hyperfine structures were analyzed as due to the ring nitrogen and two pairs of two equivalent protons. Type II radical was obtained in benzene but its life time was very short and it was, therefore, observed only immediately after irradiation.

6-Methyl-4-NQO: Both types of radicals were obtained in dioxane, while no radical was found in benzene unlikely to the case of 4-NQO. The signals of the type I (Fig. 1, b) was not affected by ^{15}N-replacement of nitro group nitrogen (Fig. 2, b) nor D-replacement of hydrogen-2 and -3, whereas the signals of the type II, were changed from triplet to doublet by ^{15}N-replacement of nitro group nitrogen.



(a)-1 (a)-2 (b)-1 (b)-2

Fig. 3. Hyperfine Structures obtained from 3-Methyl-4-NQO and 5-NQO

Isotope replacement affected the signal pattern in quite different manner from 4-NQO.

(a)-1: 3-methyl-4-NQO
(a)-2: 2,3-deutero-3-methyl-4-NQO
(b)-1: 5-NQO
(b)-2: 2,3-deutero-5-NQO

7-Methyl-4-NQO: Type I radical (Fig. 1, c) was obtained in dioxane. The spectrum, which was not affected by ^{15}N-replacement of nitro group nitrogen, was analyzed as being due to spin coupling with the nitrogen and two equivalent protons. Type II radical was obtained in benzene, the spectrum of which was changed from triplet to doublet by ^{15}N-replacement of nitro group nitrogen (Fig. 2, d).

6-Chloro-4-NQO: Both types of radicals were obtained in dioxane. The signal of the type I (Fig. 1, e) was analyzed as due to spin couplings with the ring nitrogen and one proton situated at the ortho-position to it. The spin coupling of meta-hydrogens could not be observed.

7-Chloro-4-NQO: Radical of type I (Fig. 1, f) was obtained in dioxane, its spectrum being analyzed as due to spin coupling with the ring nitrogen and two equivalent protons. Type II radical was obtained in benzene. This solvent-dependent character in radical production of this compound is entirely similar to 4-NQO.

4,8-Dinitroquinoline 1-oxide: No ESR signal could be observed.

3-Methyl-4-NQO: Among the 4-NQO derivatives examined in the present study, 3-methyl-4-NQO underwent an exceptional photodegradation reaction as well as 4,8-dinitroquinoline 1-oxide. It produced only one sort of radical both in dioxane and in benzene, the structure of which was surely different from either radical of the type I or II. Thus, although ^{15}N-replacement of nitro group nitrogen did not affect the signal pattern as in the case of type I radical, the spectrum was remarkably changed by D-replacement of hydrogen-2 and methyl hydrogens at position-3 as shown in Fig. 3. These exceptional behaviors of this compound will be discussed later in connection with its physicochemical properties.

Photodegradation of Various Quinoline Derivatives Other than 4-Nitroquinoline 1-Oxides——No stable free radicals were produced by photoirradiation of any of the compounds included in group C in either dioxane or benzene solutions. Thus, most of the compounds (4-nitroquinoline, 3-nitroquinoline 1-oxide, 8-nitroquinoline 1-oxide, 4,8-dinitroquinoline 1-oxide, 4-hydroxyaminoquinoline 1-oxide, 4-aminoquinoline 1-oxide, 4-chloroquinoline 1-oxide, quinoline 1-oxide, and quinoline) did not give ESR signals at all. Although some compounds (4-nitropyridine 1-oxide, 6-nitroquinoline 1-oxide, and 7-nitroquinoline 1-oxide) gave very weak signals, they were too diluted to be identified as such stable radicals as those produced from 4-NQO derivatives. UV-irradiation of dioxane solution of 4-HAQO which contain the free radicals produced from the compound by air oxydation[*4], decreased the signals present in the original sample and then erased them. It can be concluded therefore, that by the photoirradiation no stable radical is formed from 4-HAQO.

[*4] The oxidative radical formation from 4-HAQ Owas already reported[27] in part and will be reported in details in near future.

An interesting result was obtained by photoirradiation of 5-nitroquinoline 1-oxide listed in group B of Table II, the spectra being shown in Fig. 3. This is the only one exception, besides 4-NQO derivatives, where a stable free radical was proved in the present study. Thus, this compound gave strong ESR signals in either dioxane or benzene solutions by UV-irradiation. The signal pattern was completely same in these two solvent systems. In addition to ^{15}N-replacement of nitro group nitrogen, D-replacement at position-2 and -3 did affect the signal pattern seriously. This means that the unpaired electron of the radical produced is delocalized over the whole of this molecule. This photochemical behavior of 5-nitroquinoline 1-oxide is surely different from those of 4-NQO derivatives.

Discussion

4-NQO derivatives were shown to undergo characteristic radical formation reactions, the features being summarized as follows :

i) Two different free radicals, type I and II, were photoinduced in dioxane or benzene solutions. The unpaired electron of the former radical was proved to be delocalized mainly on the ring nitrogen and the benzene moiety of the molecule, whereas that of the latter was mostly localized on the substituent nitro group nitrogen, very large couplings of about 28 gauss being observed with the nitrogen nucleus.

ii) These radical formations are dependent on the solvent nature. Type I radicals were produced in dioxane solutions in most cases, while type II radicals were produced in dioxane or/and benzene.

iii) The production of type II radicals was inhibited by degassing of the sample at an extent of 10^{-4} mm. Hg.

It is strongly suggested from the result obtained in present ESR study that the photochemical behavior summarized above is characteristic only of 4-NQO derivatives. Among the 4-NQO derivatives examined, however, there was found two exceptions in cases of 3-methyl-4-NQO and 4,8-dinitroquinoline 1-oxide. 3-Methyl-4-NQO did not give two kinds of radicals and, moreover, the radical produced was characterized neither type I nor II. But the chemical or electronic structure of this compound is expected to be considerably changed from those of other 4-NQO derivatives. Thus, the mesomeric interaction between 4-nitro group and the aromatic ring should be strongly hindered by steric bulkiness of 3-methyl group. This was experimentally proved by UV and NMR data of this compound. UV maximum in the longer wave region shifted by 18 mμ to the shorter, and in its NMR spectrum the proton-5 resonated at about 1 p.p.m. higher field than those of other 4-NQO derivatives where their proton-5′ are suffered from a strong paramagnetic anisotropy effect of 4-nitro group[6]. These facts indicate that nitro group in this molecule must be considerably twisted out of the plane of the aromatic ring.

With regard to the other exceptional case of 4,8-dinitroquinoline 1-oxide, where no ESR signal was observed, it is expected that by substitution of another nitro group, the changes in the electronic structure is too large compared with 4-NQO-analogous compound. It may be finally concluded, therefore, that this type of radical production is a specific feature of 4-NQO analogous compounds, that is, those which have quinoline ring, N-oxide group and a nitro group at position-4 in their chemical structures and which, moreover, are not so much perturbed electronically. It is worth to note in connection with the carcinogenicity on the skin of animals that carcinogenic activity have been demonstrated only with 4-NQO derivatives listed in group A, but not with the compounds in group B nor C.

It is to be noted at the conclusion of this paper that the present discussions were made from a viewpoint of radical formation and no consideration was given to the photodegradation processes themselves which is supposed to be very complicated.

Almost all compounds examined were completely decomposed by UV-irradiation to produce a variety of unidentified materials.[*5] The role of the radical formation in these photochemical processes is still unknown, which are now under investigation.[*6]

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Summary

UV-irradiation of quinoline 1-oxide derivatives were studied by electron spin resonance technique and 4-nitroquinoline 1-oxide (4-NQO) derivatives except for 3-methyl-4-NQO and 4,8-dinitroquinoline 1-oxide were found to produce two kinds of stable radicals in dioxane or in benzene. This photochemical behavior was discussed from a view point of radical formation.

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[*5] Kosuge *et al.* isolated several quinoline derivatives from the degradation products of 4-NQO.[28]

[*6] Photochemical rearrangements of aromatic amine N-oxides have been recently studied.[29~35] See following references and those cited therein.

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