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30. Oxidation Reaction of Carcinogenic 4-Hydroxyaminoquinoline 1-Oxide.*3

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An amphoteric compound 4-hydroxyaminoquinoline 1-oxide(4-HAQO) was oxidized by oxygen to lose one electron from NHOH group of the molecule and to produce a stable free radical. This oxidative radical production was accelerated by addition of several kinds of oxidants. The UV-absorption of the free radical thus produced, showed its maximum at 455 mμ. On the other hand, the oxidation of 4-HAQO was protected by the presence of such a reducing agent as ascorbic acid. As a result, the presence of such a reducing agent made it possible to record the UV spectrum of 4-HAQO in alkaline media.

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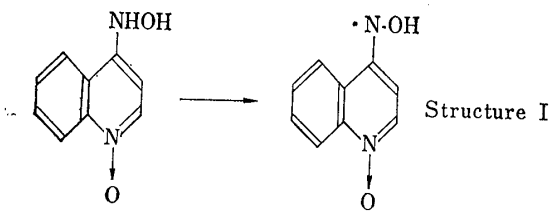
4-Hydroxyaminoquinoline 1-oxide (4-HAQO) is an amphoteric compound, so that it dissolves in either acidic or alkaline solvents whereas it is almost insoluble in neutral water. As we described in the previous papers,[1,6] its alkaline solution gets easily colored and gives various decomposed products, although it is stable in acidic media. It was suggested by several authors[2] that oxidation of this compound occurred very easily in alkaline media to give unstable oxidation products such as 4-nitrosoquinoline 1-oxide, etc.

In the course of our serial studies on the chemical and biochemical reactivities of carcinogenic quinoline derivatives, we found that 4-HAQO, one of the potently carcinogenic agents,[3~5] gave strong ESR signals in alkaline media and in some organic solvents. We determined the chemical structure of the free radical produced in dioxan as structure I from the analysis of its ESR spectra with a help of the isotope replacement technique and from the theoretical consideration by using the unrestricted SCF molecular orbital method.[6] Thus, 4-HAQO was found to be oxidized by one electron step to lose one hydrogen atom from -NHOH group in the molecule, an odd electron delocalizing all over the π-electron system. It will be emphasized in this paper that this free radical must be produced at the initial stage in the oxidative degradation process of 4-HAQO. This paper describes the details of the one electron step-oxidation of this molecule and protection of the oxidation by such reducing agents



Structure I

*1 Tsukiji 5-Chome, Chuo-ku, Tokyo (片岡順子, 今村 詮, 川添 豊, 千原呉郎, 永田親義).
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*3 This paper constitutes part V of a series entitled "ESR Studies on 4-Nitroquinoline 1-Oxide and its Related Compounds."

1) C. Nagata, N. Kataoka, A. Imamura, Y. Kawazoe, G. Chihara : Gann, **57**, 323 (1966).
2) T. Kosuge, M. Yokota : Yakugaku Zasshi, **85**, 69 (1965).
3) H. Endo, F. Kume : Naturwiss., **50**, 524 (1963).
4) H. Endo : Gann, **54**, 443 (1963).
5) Y. Shirasu, A. Ohta : *Ibid*, **54**, 221 (1963).
6) N. Kataoka, A. Imamura, Y. Kawazoe, G. Chihara, C. Nagata : Bull. Chem. Soc. Jap., in press.

as ascorbic acid in alkaline media. Discussions will also be given to the ultraviolet (UV)-absorption of the free radical thus produced.

Experimental

Materials——4-HAQO hydrochloride was prepared by catalytic reduction of 4-nitroquinoline 1-oxide with Pd-charcoal and purified by recrystallization from 20% aqueous hydrochloric acid (m.p. 193~196°).[7,8] The reagents used, hydrogen peroxide, ferric chloride, potassium permanganate, ascorbic acid, phenylhydrazine, cystein, mercaptoethanolamine, were all commercially available reagents in the guaranteed reagent (G.R.) grade. Benzoyl peroxide was of E.P. grade. Dioxan for solvent was purified as follows : After distillation of commercially available reagent of G.R. grade, sodium wire was added to it to decompose dioxan-peroxide. The buffer solution used were Britton Robinson Buffer.

ESR Measurement——ESR measurement was carried out at a concentration of 7.5×10^{-5} to 7.5×10^{-4} Mol. of 4-HAQO in a quartz tube (5 mm. in diameter for dioxan solutions and 0.75 mm. for polar solvent solutions) by a Japan Electron Optics Laboratory's JES-3BX spectrometer with 100 Kc/s field modulation.

UV Measurement——UV spectra were recorded on a Cary-14 spectrometer. Each aqueous sample solution contains 4-HAQO at a concentration of 7.5×10^{-5} Mol.

pH Measurement——pH measurements were carried out by Metrohm Herison Potentiometer Model E-336.

Results and Discussion

Oxidative Radical Formation from 4-HAQO

As previously reported,[1] 4-HAQO free base and its hydrochloride undergo a dehydrogenation reaction in the presence of oxygen in dioxan and also in alkaline aqueous media (at higher pH values than 9) to produce a stable free radical of the structure I. On the other hand, this compound gave rise to no free radical in neutral or weakly alkaline aqueous media, and neither in acidic media in either aqueous or organic solvents. This was proved by ESR measurements as shown in Fig. 1. Fig. 2 and Fig. 3 show the electron spin resonance (ESR) spectra which were obtained when hydrogen peroxide or benzoyl peroxide was added to the solution examined. The presence of benzoyl peroxide or hydrogen peroxide clearly enhanced the ESR signal intensity of the radical in the dioxan solution (Fig. 2). Further, as is seen in Fig. 3a the production of the free radical was seen even in the acidic dioxan by adding the hydrogen peroxide. Similarly, the presence of these peroxides promotes the oxidation of 4-HAQO in acidic methanol solution, too (Fig. 3b), although hydrogen peroxide did not bring about even a slight increase in the signal intensity in acidic aqueous solution (Fig. 3c). These experimental results show that the free radical production is accelerated by the presence of peroxides.

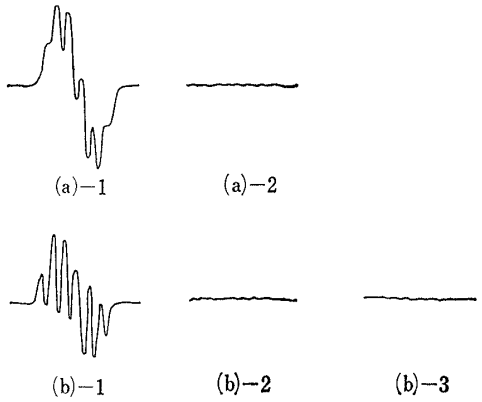

Fig. 1. Free Radical Production from 4-HAQO by O_2-Oxidation
(a)-1, dioxan solution : Few minutes after it was dissolved.
(a)-2, acidic dioxan solution
(b)-1, alkaline aqueous solution
(b)-2, neutral aqueous solution
(b)-3, acidic aqueous solution

As other oxidative agents, ferric chloride or potassium permanganate etc. were used in stead of peroxides. Ferric chloride was effective in acidic aqueous media (pH 1.8) and a considerable amount of the ion was necessary for a remarkable signal increase (Fig. 4). Only in neutral condition the acceleration of radical production by ferric chloride or potassium permanganate in dioxan was observed (Fig. 5).

7) E. Ochiai, T. Naito : Yakugaku Zasshi, **64**, 206 (1944).
8) E. Ochiai, A. Ohta, H. Nomura : This Bulletin, **5**, 310 (1957).

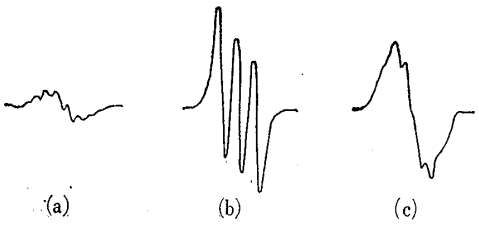

Fig. 2. Acceleration of Raidcal Production in Dioxan by Peroxides

(a) no addition
(b) soon after the addition of hydrogen peroxide (excess)
(c) soon after the addition of benzoyl peroxide (excess)

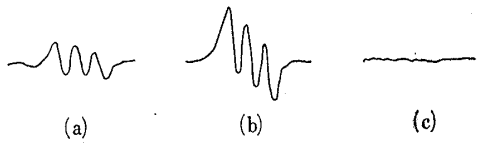

Fig. 3. Free Radical Production in Acidic Media by Adding Hydrogen Peroxide

(a) in dioxan (b) in methanol
(c) in water; In all cases, the measurements were carried out few minutes after adding hydrogen peroxide (excess).

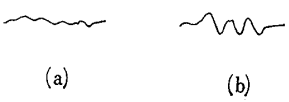

Fig. 4. Free Radical Production in Acidic Aqueous Media by Ferric Chloride : pH 1.8

(a) soon after the addition of ferric chloride in the same amount as 4-HAQO
(b) soon after the addition of ferric chloride in three folds of 4-HAQO

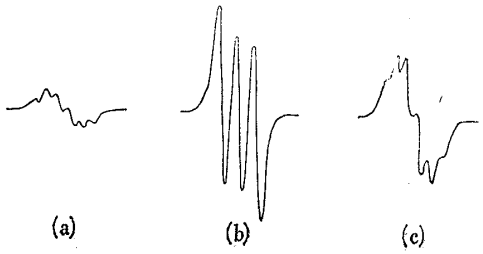

Fig. 5. Acceleration of Free Radical Production in Dioxan by Ferric Chloride or Potassium Permanganate

(a) no addition
(b) soon after the addition of ferric chloride in the same amount as 4-HAQO
(c) soon after the addition of potassium permanganate in the same amount as 4-HAQO

To be interesting, ferrous and cuprous ions did accelerate catalytically the radical formation reaction more effectively than ferric and cupric ions. The details on the metal-catalized oxidation will be reported in a forthcoming paper.

It should be mentioned, furthermore, that this oxidation does not involve photo-excitation of the molecule in the course of dehydrogenation process. Thus, ESR signal was observed under the dark reaction condition and, when irradiated with ultraviolet (UV)-lamp, the radical thus produced was decomposed quickly.

Now, the causal relation between the formation of the free radical and the decomposition of 4-HAQO molecule was examined by comparing the increase in ESR signal intensity with the decrease in UV-absorption of 4-HAQO. Well parallelism was found between them with regard to the dependence upon pH of the solution, reaction time, co-existence of some reagents and so on. The fact that the radical concentration in the solution examined could become more than a quarter of the original concentration of the 4-HAQO molecule leads an important suggestion that this free radical is an intermediate derivative at the initial stage of the oxidative degradation of 4-HAQO.

Protection of Oxidation of 4-HAQO by Addition of Reducing Agents

Since decomposition of 4-HAQO in alkaline media was suggested to be initiated by the oxidative radical formation, 4-HAQO is expected to be stabilized in the presence of appropriate reducing agents as the antioxidant even in strongly alkaline media. In fact, ascorbic acid worked effectively as an antioxidant as shown in Fig. 6.

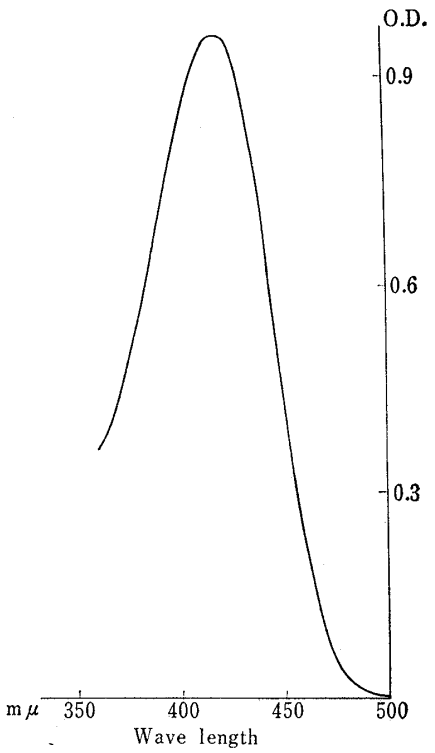

Fig. 6. Ultraviolet-Absorption of 4-HAQO in Alkaline Aqueous Solution in the Presence of $4\times10^{-4}M$. Ascorbic Acid : pH 11.6

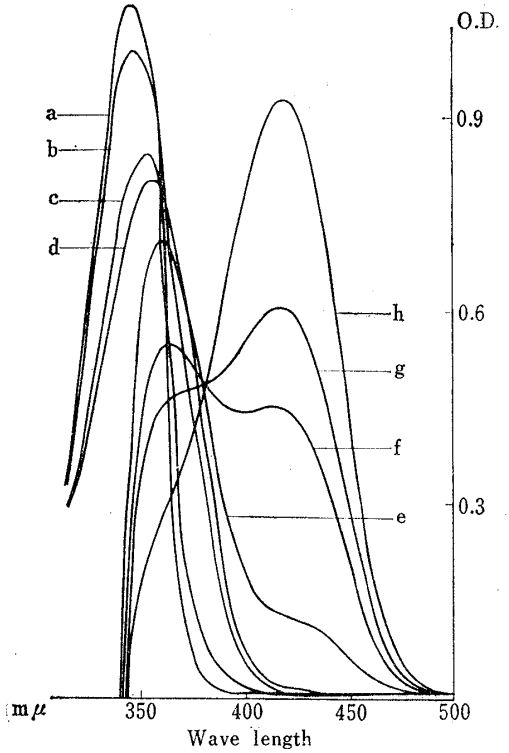

Fig. 7. Ultraviolet-Absorption of 4-HAQO in Aqueous Media at Various pH Values

In alkaline condition, appropriate ascorbic acid are present.
pH values: (a) 2.00, (b) 3.00, (c) 4.12, (d) 6.98, (e) 8.48, (f) 9.25, (g) 9.6, (h) 10.7

Addition of 2 to 5 folds amount of ascorbic acid to the aqueous alkaline or dioxan solutions of 4-HAQO protected the oxidation of 4-HAQO and in aqueous media it gives a UV-absorption at the longer wave region around 418 mμ which was not changeable. This solution did not gave ESR signal at all. But after a while, UV-absorption at 418 mμ began to decrease and at the same time the ESR signal appeared and it grew intense. These facts indicate that 4-HAQO was protected from oxidation in compensation for oxidative degradation of ascorbic acid molecule. Addition of ascorbic acid erased the ESR signal even after a large amount of the free radical was formed, because probably of reduction of the free radical to 4-HAQO as shown in Chart 1. The molecule of ascorbic acid added must be consumed for the reduction not only of the oxidant present in the

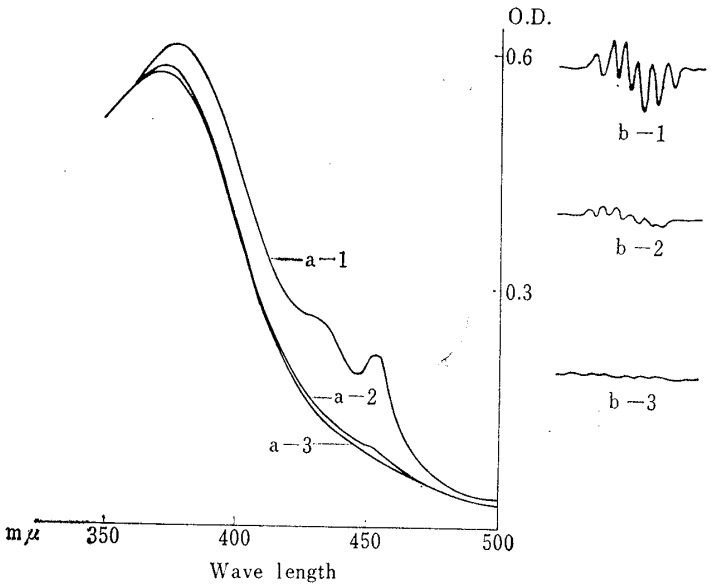

Fig. 8. Spectral Change Depending on Time

(a) UV-spectra (b) ESR-spectra
(1) Soon after 4-HAQO was dissolved
(2) after 6 minutes (3) after 12 minutes. pH 11.4.

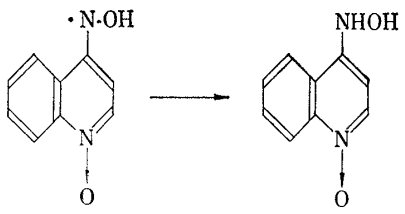

Chart 1*4

solution but also of the free radical produced. As described above, the UV-absorption spectra were recorded of the amphoteric 4-HAQO in alkaline media in the presence of ascorbic acid. The UV-absorption maximum was moved to 418 mμ ($\varepsilon=1.36\times10^4$) from 345 ($\varepsilon=1.45\times10^4$) and 355 mμ ($\varepsilon=1.08\times10^4$) in acidic and neutral media, respectively.

These spectra were reproduced in Fig. 7. In this figure,

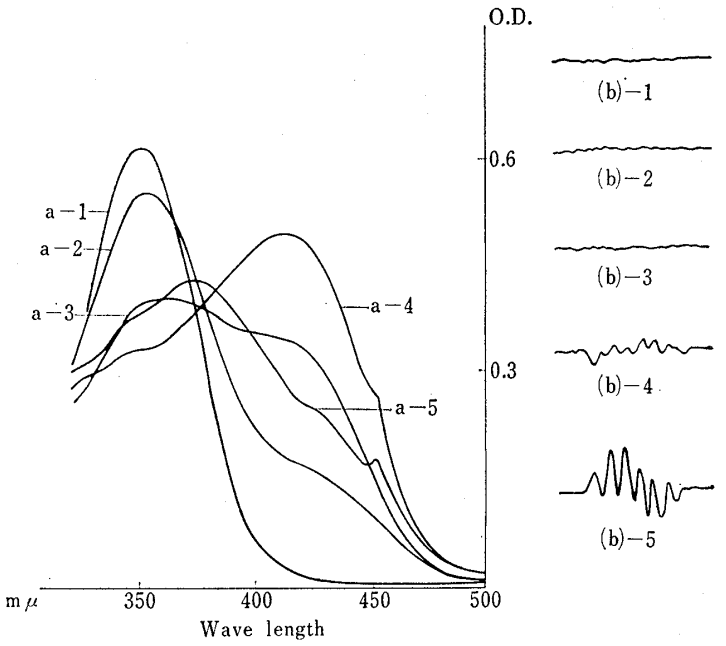

Fig. 9. Spectral Change Depending on pH

(a) UV-spectra (b) ESR-spectra
pH values; (1) 7.33, (2) 8.68, (3) 9.45, (4) 10.32, (5) 11.61, at the concentration of 5.7×10^{-5}

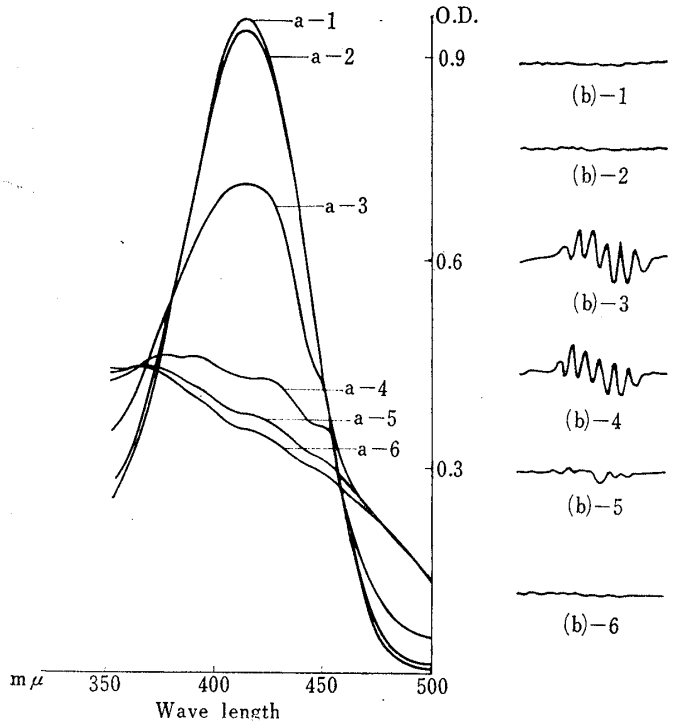

Fig. 10. Spectral Change Depending on Time in the Presence of Ascorbic Acid

(a) UV-spectra (b) ESR-spectra
(1) Soon after 4-HAQO was dissolved.
(2) after 10 minutes (3) after 20 minutes
(4) after 30 minutes (5) after 38 minutes
(6) after 45 minutes. pH 11.06

amphoteric nature of 4-HAQO was clearly proved because two isosbestic points were seen there.

Other reducing agents such as phenylhydrazine, mercaptoethanolamine, cystein, etc. could take the place of ascorbic acid, although they were less effective than ascorbic acid.

The UV-Absorption of the Free Radical Oxidatively Produced

When 4-HAQO was dissolved in alkaline media (at pH 11) without addition of ascorbic acid, an additional absorption peak appeared at 455 mμ as shown in Fig. 8. This peak was observed only for a few minutes after 4-HAQO was dissolved. This absorption peak was assigned as the free radical produced from 4-HAQO which was determined as the structure I. Thus, this band could be observed only in the solutions at the pH regions where the ESR signal could be

*4 Hozumi, *et al.* have found that 4-HAQO oxidized SH-group of glutathion only in the presence of oxygen. (by private communication). This is considered to be due to such hydrogen abstruction by free radical produced from 4-HAQO.

detected (Fig. 9). Another evidence was that, in the presence of ascorbic acid in the solution examined, neither ESR signal nor UV-absorption at 455 mμ was observed. After a while the UV-absorption at 455 mμ appeared and at the same time ESR signal also became to appear (Fig. 10). In addition to this, the UV-absorption coefficient at 455 mμ began to diminish rapidly and in company with this, ESR signal was diminished.

This free radical thus obtained is an interesting intermediate compound on the oxidation process of 4-HAQO since it is considerably stable even in protic solvents at room temperature. This might be also of interest in connection with the role of 4-HAQO in its carcinogenicity on the skin of animals.

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