Mem. Fac. Educ., Shimane Univ. (Nat. Sci.) Vol.23－No.1, pp.35－41, July. (1989)

ESR and UV Absorption Spectra of Amino Azobenzene Derivatives

Kunihisa SOGABE,* Akemi ARAKAWA** and Ikko SAKAMOTO*

ABSTRACT: UV and Visible absorption spectra of amino azobenzene and dimethyl amino azobenzene showed the similar behaviors under variations of PH, substituents, and oxidants. The maximal absorption band around 350 nm seen in both amino azobenzenes was found to relate closely with the quarterization by the proton addition or the N-hydroxylation by the oxidation, of the amine substituent. The fact that an ESR spectrum of azobenzene aminyl-1-oxy radical is observed in oxidized amino azobenzene suggests a possibility for N-hydroxylation of amino azobenzene under oxidation and to involve the same pathway as the metabolic activation of carcinogenic dimethyl amino azobenzene.

INTRODUCTION

Many azo compounds such as amino azobenzene, dimethyl amino azobenuene, or phenyl triazenes are widely used as dyes because azo compounds containing cojugated systems of double bonds have the property of selectively absorbing ultraviolet light. Especially, dimethyl amino azobenzene, butter yellow, in well known as one of the oledest carcinogenic compounds and formed an excellent stuff for the research of metabolic pathway.[1] Nagata, et. al.[2] have investigated the correlation of the concentration and kinds for carcinogenic amines accumulated in rat livers. However, no informations on their metabolic activation, or their behaviors under PH variation or oxidative reaction for other azo compounds such as azobenzene or triazene are reported.

Forrester, *et. al.*,[3] and Miller, *et. al.*,[4] separately reported the mechanism on metabolic activation of carcinogenic amines such as N-hydroxy amino fluorene, in which the formation of nitroxide radicals is a key step and a significant effect on this activation pathway is easily undergone by PH variation or oxidation. ESR spectra of nitroxide radicals produced in various hydroxamic acids or hydroxyl amines under oxidation, UV-irradiation, and PH variation were observed and discussed on their actdvation process.[5]

A proton addition to amino group of carcinogenic aromatic amines would produce a

* Dept. of Chemistry, Fac. of Education, Shimane University, 1060 Nishikawatsu-cho, Matsue 690

** Present address : 954-3 Goutsu-cho, Goutsu 695

considerable change in an electron transfer sequence of a given conjugated system, producing structural variations of an amine moiety. In order to solve the correlation between the structure and reactivity of metabolites such as amino azobenuene derivatives, ESR and UV absorption spectra were observed at the same time under oxidative reaction or PH variation.

EXPERIMENTAL

Azo compounds (azobenzene, amino azobenzene, dimethyl amino azobenzene, and diphenyl triazene) were purchased from Tokyo Kasei Co., and used without further purification. Each azo sample was dissolved in fresh dioxane by *ca.* 10^{-2} mol/l and its solutio sample was stored below 0°C. UV and visible spectra were recorded by Hitachi 220 UV Double Beam Spectroscopy (185 - 900 nm) with 10 mm quartz flow cells at room temparature. Glass pump (EYELA) was built up for flow system. PH control was carried out with PH meter (Horiba) and dilute HCl solution. ESR Spectra wer recorded by JEOL FElX ESR Spectroscopy at room temparature. 500 W Xe lamp made by WKCON was used as UV source and UV light was directly irradiation on the sample tube in the ESR cavity. ESR parameters of g values and hyperfine coupling constants were measured utilizing a mangne standard sample.

RESULTS AND DISCUSSION

UV and visible absorption spectra of azobenzene and two amino azobenzene-dioxane solutions measured at PH = 7 are characterized by their maximum absorption band below 250 nm and strong maximum absorption at 310 (ε = 3.4), 380 (ε = 2.9), and 410 nm (ε = 2.

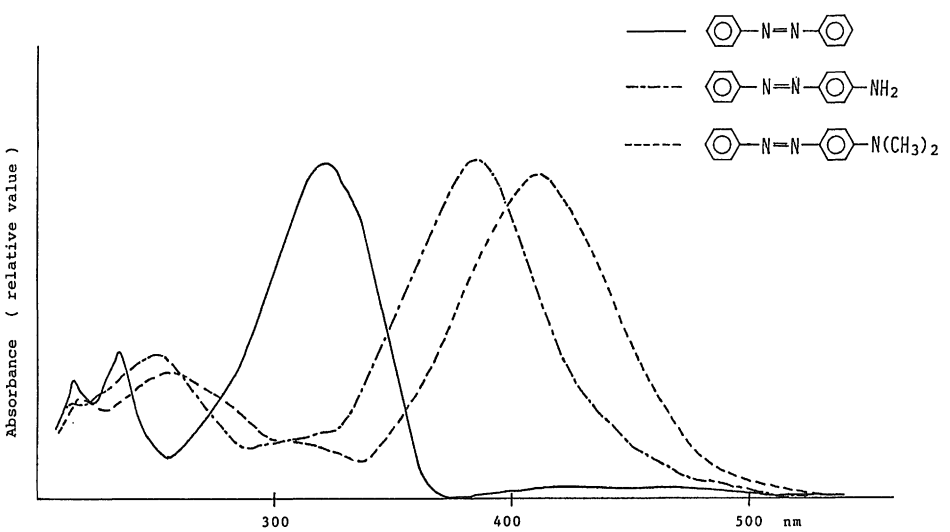


Figure 1. Absorption spectra of (a) azobenzene, (b) amino azobenzene and (c) dimethyl amino azobenzene observed at PH = 7.

8 $mol^{-1} \cdot dm^{-3}$) for azobenzene, amino azobenzene, and dimethyl azobenzene, respectively, as shown in Figure 1. These absorption patterns are resemble closely except the difference in the the position of their strong maximum absorption bands which they may be resulted from a π-π^* transition between π bonding energy levels in azo group and in phenyl rings. The absorption band at 310 nm is characteristic of azobenzene and is shifted to longer wave lenghs, 380 or 410 nm amino azobenzene or dimethyl amino azobenzene respectively, by an effect of a subntituent such as amino or dimethyl amino group with the electron donating ability. The introduction of the powerful substituent leads to the extention of the electron transfer sequence in the conjugated double bond from azobenzene to both amino azobenzenes.

The absorption band in the wave length should be calculated as follows;[5]

$$\lambda = \frac{8mCL^2}{h(N+1)}$$

where m is an electron mass, and L is length of a given conjugated system, and N is a number of double bond, and h is Planp's constant, and λ is a wave length of an energy level corresponding to a π-π^* transition. the maximum absorption band in the wave length for the amino azobenzene was calculated as about 350 nm by taking account of the electron transfer sequence with N = 8, length r_c = 0.139 nm, and total length L = 0.973 nm. This calculated value is in fair agreement with the observed value and the error can be greatly diminished by the correction for the length of the electron transfer sequence, L.

The functional groups such as NH_2 and OH are usually called the auxochromes against the chromophores including the azo group. p-Hydroxy azobenzene is expected to have the similar conjugated system and structure with the amino azobenzene, and to be an excellent stuff to compare the effect of the substituent on the absorption band. The absorption spectrum of p-hydroxy azobenzene measured at PH = 7 exhibits the maximum at 350 nm,

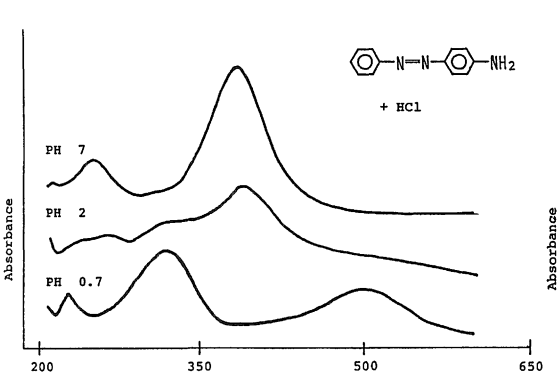


Figure 2. Absorption spectra of amino azobenzene observed at (a) PH = 7, (b) PH = 2.0, and (c) PH = 0.7, respectively.

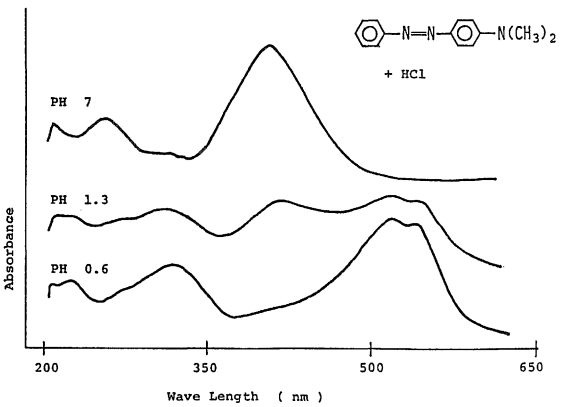


Figure 3. Absorption spectra of dimethyl amino azobenzene observed at (a) PH = 7, (b) PH = 1.3, and (c) PH = 0.6, respectively.

in which this experimental value is in good agreement with the value calculated theoretically for the amino azobenzene. The difference in the maximum adsorption bands between amino azobenzene and p-hydroxy azobenzene shows the electron transfer sequence including the hydroxy group is considerablly weaker than that including the amino group.

This result seems to be ardsed from the difference in the mobility of the pair electrons and especially reflect such situation for the pair electrons bound to oxygen atom, that is, a mutual repulsion betwees two electron pairs becomes evident and allows to a decay of an electron distribution in the conjugated system as four pair electrons belonged to oxygen atom are brought closely together that their electron clouds begin to overlap. Also this fact is closely consistent with the order of the substituent effect os the maximum absorption band the so-called spectrochemical series[6];

$$-OH < -NH_2 < -N(CH_3)_2$$

Such the electron transfer sequence including amino group found in both amino azobenzenes is expected to be readily modified by thier PH or structural alternations. Indeed, slight band shift is recognized by the functional group effect as found in Figure 1. Interesting spectral changes of amino azobenzene and dimethyl amino azobenzene under severe PH variations are shown in Figures 2 and 3, respectively. The drop in PH leads to the decrease in the intensity of the strong absorption band (380 or 410 nm) characteristic for two amino azobenzenes and especially, below PH = 2, new absorption bands at 320 and 500 nm for amino azobenzene and at 320 and 550 nm for dimethyl amino azobenzene appear as can be seen from Figs. 2 (c) and 3 (c).

The absorption band at 320 nm found in both amino azobenzenes is readily interpreted as a shift back from the thier maximum absorptiom band which was already shifted to around 400 nm by the introduction of the amino group. Such the shift back may be arised by the decrease in the electron supply from the amino group to the conjugated system on the addition of the proton. In the case of p-hydroxy amino azobenzene, the proton addition also results in the blue shift of the absorption band from 350 to 320 nm, showing the substituent effect on the band shift. Magnitude in the blue shift, the spectrochemical series, should depend on the coordination power of the pair electrons against the proton, relating to the mutual repulsion between the pair electrons.

The absorption bands around 400 nm appeared by the proton addition to both amino azobenzenes are interpreted to partially split into two bands; one is the band shifted to around 320 nm mentioned above, and the other is a newly appeared band around 500 nm which is identified to result from a n-π^* transition. The interesting fact that this n-π^* band is observed in both amino azobenzenes but azobenzene may suggest an important role of a functional substituent such as amino group. The appearance of the n-π^* transition seems to imply a formation of a quarternary amine by the proton addition and this is closely associated with pair electrons in the nonbonding energy level becoming free from the conjugated system.

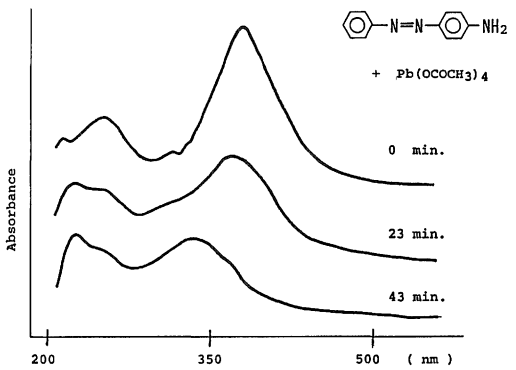


Figure 4. Absorption spectra of amino azobenzene oxidized with $Pb(CH_3COO)_4$. Spectra were recorded at (a) 0 min., (b) 23 min., and (c) 43 min. after mix..

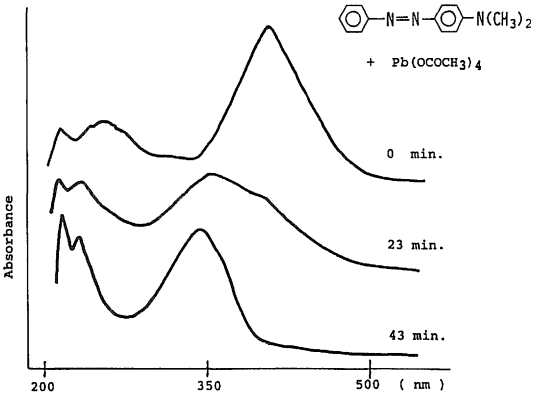


Figure 5. Absorption spectra of dimethyl amino azobenzene oxidized with $Pb(CH_3COO)_4$. Spectra were recorded at (a) 0 min., (b) 23 min., and (c) 43 min. after mix..

The n-π^* transition absoption spectra observed in the acidic solutions of both amino azobenzenes may suggest a possibility for an existence of a different structure, N-hydroxyl amine, under oxidative reaction.

N-Hydroxylation of amines is well known to be a key step in metabolic activations of carcinogenic aromatic compounds,[3,4] and is expected to find in the oxidative reaction of these amino azobenzenes. To investigate the metabolic activation for carcinogesic amines under oxidation as well as PH variation, absorption spectral changes of both amino azobenzenes are measured and shown in Figures 4 and 5, respectively. No absorption band around 500 nm due to the n-π^* transition is observed in these figures, while the similar bands around 340 nm due to the π-π^* transition are appeared as those shown in severe acidic solution. The appearance of the bands around 340 nm is interpreted as a result of a decrease in the substituent effect, in which the pair electrons os amino group are attracted to an oxiges atom and reduced the electron to the conjugated system.

Furthermore, the reduction of electron distribution in the conjugated system is confirmed by the lack of the band around 500 nm in these spectra of oxidized amino azobenzenes. These results seem to suggest that an attack of oxygen atom to the amine group produces similar conjugated system between protonated and oxidized amino azobenzenes, although cherical structuren betwees quarternary amine and N-hydroxyl amine are different. This result also seems to suggest a possibility of different pathway for an activation process of amino azobenzenes.

ESR spectroscopy is a powerful technique for a detection of nitroxide radicals produced in oxidation processes of various N-hydroxyl amines,[7] and this technique is applied to detect for the step of the N-hydroxylation under oxidation of amino azobenzenes. An ESR spectrum is observed in oxidized amino azobenzene as shown in Fiqure 6, while no spectrum is

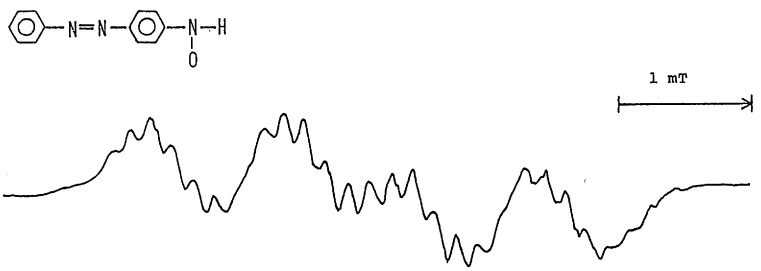
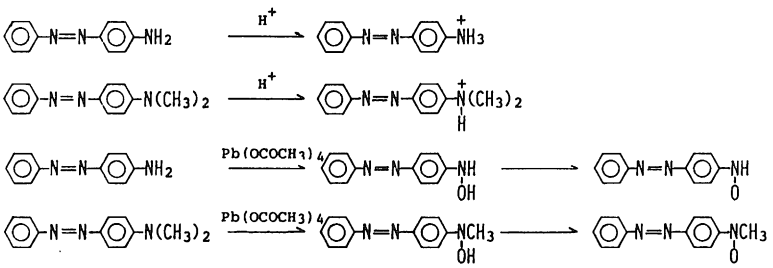


Figure 6. ESR Spectrum of azobenzene aminy-1-oxy radical produced in oxidized amino azobenzene with $Pb(CH_3COO)_4$.

observed in oxidized dimethyl amino azobenzene in spite of many attempts. This ESR pattern is very similar with that of N-phenyl nitroxide radical,[8] and the radical obtained here is easily identified as azobenzene aminyl-1-oxy from an analysis of the spectrum shown in Figure 6. This shows that the azo group bound by two phenyl is considerablly strong against the oxidation as well as the PH variation, whereas the triazene group ($-N-N=N-$) bound by two phenyl rings was apt to release the azo group on PH variation or oxidation.[9]

On the other hand, the amino group is easily attacked by an oxygen atom or a proton, forming a hydroxyl amine or a quarternary amine, respectively. An observation of azobenzene aminyl-1-oxy radical seems to suggent a formation of N-hydroxyl amino azobenzene which is further oxidized to the nitroxide radical by an oxygen atom. N-hydroxyl amine in generally produced in an oxidative process of amines such as carcinogenic β-naphthyl amine or acetyl amino fluorene, resulting in corresponding nitroxide radicals. The metabolic activation process for amino azobenzenes is supposed as follows.



Amino azobenzene shows the same behavior for PH variations as dimethyl amino azobenzene, as can be found in Figs. 1- 3 , and may be therefore undergone the same metabolic activation as dimethyl amino azobenzene, though no corresponding nitroxide radical from dimethyl amino azobenzene is detected. From this discuss, amino azobenzene may be suggested to act carcintgenic amine under mild acidic or oxidative condition, although further studies on methyl amino azobenzene or diphenyl triazene compound should be continued for a detail mechanism on the metabolic activation.

REFERENCES

1) S. Nagakura and J. Tanaka, J. Chem. Phys., **22**, 563 (1954).
2) T. Kimura, M. Kodama and C. Nagata, GANN, **71**, 417 (1980).
3) A. R. Forrester, M. M. Ogilvy, and R. H. Thomsom, J. Chem. soc. C **1970** 1081.
4) J. A. Miller, Cancer Res., **30**, 559 (1970); E. C. Miller, Cancer Res., **38**, 1479 (1978); F. F. Kadlubar, J. A. Miller, and E. C. Miller, Cancer Res., **36**, 1196 (1976).
5) J. E. Huheey "Inorganic Chemistry" Harper & Row, New york, N. Y. (1972).
6) R. S. Drago, "Physical Methods in Chemistry" Suders company, Philadelphia, PA, (1977).
7) K. Sogabe, Preprint on 21th ESR meeting, Matsuyama (1983).
8) K. Sogabe, Memo. Fac. Educ., Shimane Univ. **14**, 11, (1981).
9) K. Sogabe, Preprint on Bull. Chem. Soc. Jap. Matsuyama 461 (1985).