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REDUCTION BY A MODEL OF NAD(P)H. IX. ESR STUDY ON THE BIOMIMETIC REDUCTION OF BENZILS WITH 1-BENZYL-1,4-DIHYDRONICOTINAMIDE

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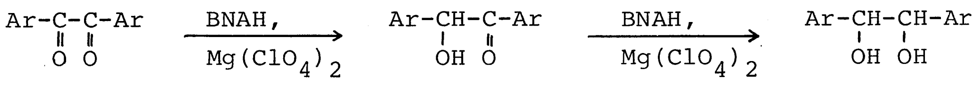
ESR studies on the biomimetic reduction of benzils by 1-benzyl-1,4-dihydronicotinamide indicate the formation of anion radicals of benzils. In the presence of magnesium perchlorate, the anion radicals are found to coordinate onto the metal ions.

On model studies of NAD(P)H-dependent biological reductions, we reported that the reduction of carbonyl compounds and olefins by 1,4-dihydropyridine derivatives as models of NAD(P)H is accelerated by metal ions[1,2] or photo-irradiation.[2,3] On the basis of ESR spectroscopy, we now wish to report informations on the mechanism of biomimetic reduction of benzils.[3]



$$\text{Ar-}\underset{\text{O}}{\underset{\|}{\text{C}}}\text{-}\underset{\text{O}}{\underset{\|}{\text{C}}}\text{-Ar} \xrightarrow[\text{Mg(ClO}_4)_2]{\text{BNAH,}} \text{Ar-}\underset{\text{OH}}{\underset{|}{\text{CH}}}\text{-}\underset{\text{O}}{\underset{\|}{\text{C}}}\text{-Ar} \xrightarrow[\text{Mg(ClO}_4)_2]{\text{BNAH,}} \text{Ar-}\underset{\text{OH}}{\underset{|}{\text{CH}}}\text{-}\underset{\text{OH}}{\underset{|}{\text{CH}}}\text{-Ar}$$

A mixture of p,p'-dichlorobenzil and 1-benzyl-1,4-dihydronicotinamide (BNAH) in dry dimethyl sulfoxide (DMSO) prepared carefully in the dark under high-vacuum (10^{-6} Torr) did not give any ESR signals.[4] After the mixture was exposed to scattered light in a room for a short period, it gave a weak ESR signal which was enhanced by further exposure to give a spectrum (g = 2.005) shown in Fig. 1-a. The spectrum is identical with that of the anion radical of p,p'-dichlorobenzil obtained in the t-BuOH-DMSO system.[5] The hyperfine structure of the spectrum is composed of 21 lines, of which 17 are attributable to the splitting by two types of aromatic protons ($\underline{a}_m = 0.39$, $\underline{a}_o = 1.06$); the others are

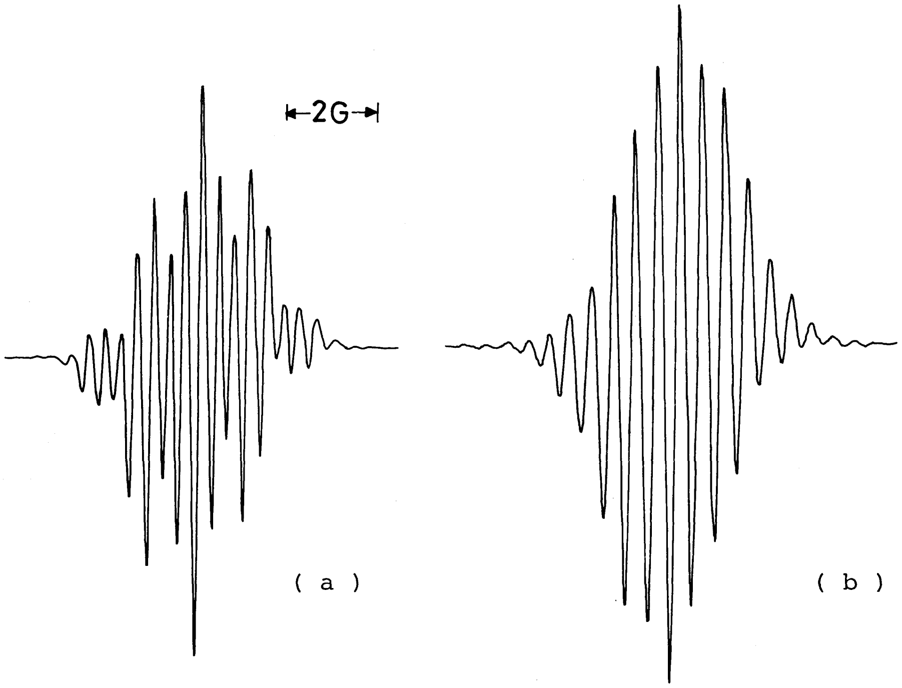
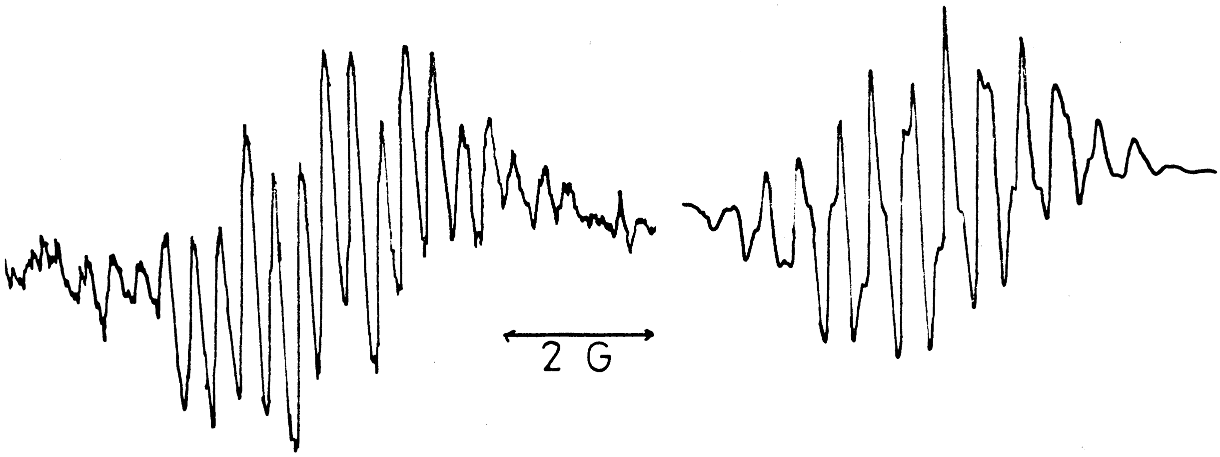


Fig. 1. ESR spectra of p,p'-dichlorobenzil anion radical in DMSO at room temperature in the absence (a) and presence (b) of magnesium perchlorate.

probably the splitting due to chlorine atoms.[6)] In the presence of magnesium perchlorate, on the other hand, a spectrum (g=2.0045) shown in Fig. 1-b was observed after the mixture was exposed to scattered light in a room for one minute, whereas no signal was recorded without exposure. The intensity of the spectrum in Fig. 1-b is stronger than that in Fig. 1-a. The spectrum in Fig. 1-a can be converted into the spectrum in Fig. 1-b by adding the magnesium salt to the system. The spectrum Fig. 1-b is composed of 19 hyperfine splittings, of which 13 lines can be ascribed to the separation with two types of aromatic protons ($\underline{a}_m$=0.48, $\underline{a}_o$=0.99). Since there is no doubt that the species which exhibit the spectrum in Fig. 1-b have no hydrogen on the carbonyl carbon, this spectrum is attributable to the anion radical of p,p'-dichlorobenzil stabilized by magnesium cation probably by chelation.[7)]

Radical species from unsubstituted benzil were observed under similar conditions: the absence of magnesium ion gave ESR signals centered at g=2.0051 as illustrated in Fig. 2-a. The spectrum is composed of splittings by aromatic protons ($\underline{a}_m$= 0.34 and $\underline{a}_o$=$\underline{a}_p$= 1.04) and is ascribed to the anion radical of



(a) (b)

Fig. 2. ESR spectra of benzil anion radical in DMSO at room temperature in the absence (a) and presence (b) of magnesium perchlorate.

benzil.[5)] The addition of the magnesium salt changed the spectrum in Fig. 2-a to other labile spectra such as that in Fig. 2-b (g=2.0045) which, unfortunately, cannot be analyzed with certainty because of the instability during the measurement of ESR spectra.[8)] However, in analogy with that of p,p'-dichlorobenzil, the spectrum may be attributed to that of benzil anion radical coordinated by magnesium ion.

Earlier, we demonstrated that the reduction of an olefin or of benzil by NADH or its model compounds is accelerated by photo-irradiation.[2,3)] Similar effect of light was also observed in the process of one-electron transfer from a model compound to methyl viologen[9)] or hemin.[10)] Thus, the reducing power, or the releasing power of one electron, of NADH and its model compounds is undoubtedly increased by photo-irradiation.[9)]

Consequently, the present result reveals unequivocally that, in the course of the reduction of an α-diketone, the model compound transfers one electron to the substrate to form the corresponding radical anion, which is stabilized[11)] by chelation with a metal ion.[12)] In other words, both light and magnesium ion assist to increase the concentration of the anion radical in the present system so that its ESR spectrum being observable.

It has also been established that the reduction, in the dark, of thiobenzophenone with BNAH involves electron-proton-electron transfer processes instead of one-step hydride transfer.[13)] Since the electron-transfer process is reversible, it seems reasonable to expect that magnesium ion functions to shift the equilibrium position to the favorable side. It is known that NAD(P)H-dependent alcohol dehydrogenase requires a cooperation of zinc ion.$^{14)}$ The present study, which we are further studying in detail, may provide non-enzymatically experimental support for this hypothesis.

References and Notes

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2) Y. Ohnishi, M. Kagami, and A. Ohno, *ibid.*, 2437 (1975).

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4) When a mixture of p,p'-dichlorobenzil (1.0 mmol), BNAH (5.7 mmol), and $Mg(ClO_4)_2$ (6.7 mmol) in acetonitrile was allowed to react for 115 hr at 50°C in a dark, the corresponding α-keto alcohol (28%) and 1,2-diol (44%) together with the α-diketone recovered (12%) were isolated.

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7) The proton hyperfine splittings are slightly perturbed by the presence of associated metal ion. For example, a_m and a_o in benzophenone sodium ketyl are 0.87 and 2.60, respectively, whereas in the magnesium ketyl they are 1.06 and 2.87, respectively. See, N. Hirota, in "Radical Ions," E.T. Kaiser and L. Eevan, Eds., Interscience Publishers, New York, N.Y., 1968, p.35.

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10) Unpublished result in our laboratory.

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