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OXYGENATION OF BIPHENYL BY THE REAGENT SYSTEM $\text{Fe}(\text{MeCN})_6^{2+}\text{-H}_2\text{O}_2\text{-Ac}_2\text{O}$:
AN IMPLICATION TO THE MECHANISM IN MAMMALIAN METABOLISM

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Oxygenation of biphenyl with $\text{Fe}(\text{MeCN})_6^{2+}\text{-H}_2\text{O}_2\text{-Ac}_2\text{O}$, a model enzyme system for mono-oxygenase, was explored in connection with mammalian metabolism. The reaction yielded 2- and 4-acetoxy derivatives in a ratio of 2.5 : 1. The mechanism of this reaction may be a laboratory model explaining the biological mechanism whereby biphenyl is oxygenated by heme-containing mono-oxygenase.

KEYWORDS — oxygenation; biphenyl; $\text{Fe}(\text{MeCN})_6^{2+}\text{-H}_2\text{O}_2\text{-Ac}_2\text{O}$; model enzyme; mono-oxygenase

Considerable interest has been focused in organic chemistry on the oxygenation of biphenyls from the viewpoints of mechanics and synthesis,¹⁾ and of mammalian metabolism.²⁾ Chemical acetoxylation of biphenyl usually affords the 2- and 4-acetoxy derivatives, preferentially the 4- compound,^{1a-e)} except for acetoxylation with $\text{Pd}(\text{OAc})_2$ which gives the 3-acetoxybiphenyl.^{1f)} Biphenyl is normally metabolized by hydroxylation to either the 2-, 4-, or both, hydroxylated derivatives. It was reported, however, that young rats hydroxylate biphenyl to both the 2- and 4-hydroxy compounds whereas adult rats preferentially form the 4-hydroxy derivative.^{2a)} Also, the *in vivo* hydroxylation of biphenyl is usually stimulated when animals are pretreated with inducing agents. For example, it has been reported that the formation of 2-hydroxybiphenyl is stimulated when rats and mice are pretreated with benzo(a)pyrene and that 4-hydroxylation is stimulated when they are pretreated with phenobarbital.^{2b)} In addition, a recent paper indicated that pretreatment with 3-methylcholanthrene caused simultaneous increases in both biphenyl 2- and 4-hydroxylation in the *in vivo* assay system with rat liver microsomes.^{2c)} It has also been reported that *in vitro* hydroxylation of biphenyl is genetically associated with the induction of aryl hydrocarbon hydroxylase (which may be cytochrome P-450) in responsive strains of mice.^{2d)} Concerning these metabolic observations, we investigated oxygenation of biphenyl by the reagent system $\text{Fe}(\text{AN})_6^{2+}\text{-H}_2\text{O}_2\text{-Ac}_2\text{O}$; AN= MeCN, a model reagent for mono-oxygenase. It afforded 2- and 4-acetoxybiphenyls in a ratio of 2.5 : 1.

Oxygenation of biphenyl by the reagent system was carried out as follows. To a solution of $\text{Fe}(\text{ClO}_4)_2$ and biphenyl in AN and Ac_2O , a solution of H_2O_2 in AN was added dropwise, keeping the temperature at $25\sim 30^\circ\text{C}$, in appropriate molar ratios ($\text{Fe}^{2+} : \text{biphenyl} : \text{H}_2\text{O}_2$) (Table I). The resulting solutions were worked up in the way reported previously,³⁾ then purified by silica gel column chromatography (CHCl_3 : hexane = 1 : 1 as an eluent) to divide the monoacetate and diacetate fractions. The separated fractions were hydrolyzed to give free hydroxylated forms and then chromatographed with silica gel to separate the 2- from the 4-hydroxybiphenyls, and the 2,2'-dihydroxy- from a mixture of 2,4'- and 2,5-dihydroxybiphenyls. Further, the 2,4'- was separated from the mixture of 2,4'- and 2,5-dihydroxybiphenyls by oxidation with Ag_2O to give the quinone (from the 2,5-dihydroxy compound) and the unchanged dihydroxy compound (i. e. the 2,4'-dihydroxy compound).

Table I. Oxygenation of Biphenyl with $\text{Fe}(\text{AN})_6^{2+}\text{-H}_2\text{O}_2\text{-Ac}_2\text{O}$ in AN

Run	Molar ratio biphenyl: Fe ²⁺ : H ₂ O ₂	Product ^{a)} (Yield, % ^{b)})					Recovery (%)
		monoacetate		diacetate			
		2-	4-	2,2'-	2,4'-	2,5-	
1	1 : 0.5 : 1.5	24.9	11.9	1.2	2.8	0.7	36.0
2	1 : 0.1 : 1.5	15.3	9.0	0.7	2.4	0.6	38.6
3	1 : 0.5 : 3.0	19.0	3.8	4.7	8.6	2.2	5.0
4	1 : 0.1 : 3.0	11.2	7.1	2.0	2.7	0.7	6.8
5	1 : 1 : 1.5	34.6	13.7	-	-	-	41.1

a) Identified by direct comparison with authentic samples.

b) Isolated yields.

In like manner, oxygenation of 2-acetoxybiphenyl with the reagent system acetoxybiphenyl : Fe^{2+} : H_2O_2 in AN in a molar ratio of 1 : 0.5 : 1.5 gave 2,2'-, 2,4'-, 2,5-diacetoxybiphenyls, 2-hydroxybiphenyl (a hydrolyzed compound), and unchanged acetoxybiphenyl in yields of 4.1, 1.8, 0.5, 7.6, and 52.3%, respectively.

Chart 1

The mechanism of the preferential formations of 2-acetoxybiphenyl and 2,2'-diacetoxybiphenyl in the oxygenations of biphenyl and 2-acetoxybiphenyl was assumed from the following observation. Absorption spectral changes were observed when biphenyl was added to a 1×10^{-4} M solution of the iron(III) solvate⁴⁾ $\text{Fe}(\text{AN})_6(\text{ClO}_4)_3$ in AN at 20°C . In these

Figure 1. Absorption spectrum change of Fe^{3+} - biphenyl in AN solution; The concentrations of biphenyl are 1×10^{-4} M (.....), 2×10^{-4} M (—). The concentration of $\text{Fe}(\text{AN})_6(\text{ClO}_4)_3$ in AN is 1×10^{-4} M (—). Mole ratio plots for the solution of $\text{Fe}(\text{AN})_6(\text{ClO}_4)_3$ -biphenyl in AN. The concentration of $\text{Fe}(\text{AN})_6(\text{ClO}_4)_3$ is 1×10^{-4} .

spectra isosbestic points existed at 310 and 400 nm (Fig. 1). This indicates the formation of a complex, weak but significant, between the iron(III) solvate and biphenyl in a mole ratio of 1 : 2 in equilibrium (assumed by mole ratio method). As already established, the reactive species of this oxygenation reagent system may be $\text{Fe}^{\text{IV}}(\text{OH})(\text{OAc})_2^{2+}$ or $\text{Fe}^{\text{IV}}(\text{OAc})_2^{2+}$, and the reaction may proceed in either an ionic or a radical mode, depending on the organic substrate.³⁾ Thus, the preferential formation of the 2-acetoxy derivative in the oxygenation of biphenyl may be due to the complex formation followed by acetoxylation with $\text{Fe}^{\text{IV}}(\text{OH})(\text{OAc})_2^{2+}$ or $\text{Fe}^{\text{IV}}(\text{OAc})_2^{2+}$, as shown in Chart 1.^{5,6)} These results may explain the *in vivo* mechanism whereby biphenyl is oxygenated by heme-containing mono-oxygenase.

On the other hand, the preferential formation of 2,2'-diacetoxybiphenyl by the reaction with 2-acetoxybiphenyl may indicate that the complex is formed either between iron(III) and the acetoxy group rather than the phenyl group, or between iron(III) and the phenyl group having electron-donating acetoxy moiety rather than the phenyl group with no substituent. Then the 2' position is acetoxylation with $\text{Fe}^{\text{IV}}(\text{OAc})_2^{2+}$.

Further, the formation of 2- and 4-acetoxybiphenyls was confirmed also in

an experiment using U- ^{14}C -biphenyl. Since the 2-hydroxy compound is a carcinogen in rats,⁷⁾ an easy supply of ^{14}C -2-hydroxybiphenyl by this synthesis method would be useful to study the biochemical mechanisms by which rat bladder tumor is induced.

References and Notes

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- 5) The major reactive species is considered to be $\text{Fe}^{\text{IV}}(\text{OAc})_2^{2+}$ in the presence of excess Ac_2O . See ref. 3).
- 6) Oxygenation of aromatic hydrocarbons is considered to proceed by an ionic mechanism. See ref. 3).
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