High Yield Hamilton System, Hydroxylation of Acetanilide Using t-Butyl Substituted Catechols

HIROMU SAKURAI* and ETSUJI HATAYAMA

Faculty of Pharmaceutical Sciences, University of Tokushima, Sho-machi 1, Tokushima 770, Japan

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Metal catalyzed oxidations of organic compounds have recently received the attention of biochemists and chemists. Many chemical systems have been studied as models for the biological oxidations catalyzed by cytochrome P-450 monooxygenases, some dioxygenases and oxidases [1] as well as to find efficient catalysts for the synthesis of phenolic compounds [2]. However, there have been few successful systems in which the structure of the models and their oxidizing activities were correlated. Catechol–Fe(III)–H_2O_2, known as the Hamilton system [3], is a suitable model for studying this problem. This paper reports the structure–activity relationship in the hydroxylation of acetanilide by t-butyl substituted catechol–Fe(III)–H_2O_2 systems which may be relevant in aiding our understanding of the hydroxylation mechanism of cytochrome P-450 monooxygenases. In connection with our finding, benzene hydroxylation was studied using several hydrophobic catechol–Fe(III)–H_2O_2 systems, but their yields were reported to be very poor when they were based on the concentration of the substrate [4].

A mixture of catechol (0.2 mmol) and acetanilide (0.1 mmol) in 10 ml of acetone was added to a mixture of 7 ml of acetone and 3 ml of 0.3 *M* sodium acetate buffer (pH 4.0) containing $Fe_2(SO_4)_3$ (0.1 mmol). The reaction was initiated by addition of 3.25 mmol of H_2O_2. The reaction mixture was stirred vigorously at 25 °C for 90 min under N_2 gas atmosphere. The oxidation products, *para(p)-*, *meta(m)-* and *ortho(o)*-acetamidophenol (AAP), were separated and quantitated by HPLC as reported previously [5].

Table I shows that the highest hydroxylation yield (6.7%) was achieved with the 3,5-di-t-butylcatechol–Fe(III)–H_2O_2 system, while 3.3% and 1.7% yields were obtained with the 4-t-butylcatechol and catechol containing systems, respectively. The yields of the 3,5-di-t-butylcatechol and 4-t-butylcatechol containing systems were approximately 4 and 2 fold, respectively, of the catechol containing system. The presence of bulky substituents on catechol may be essential for the formation of the 1:1 ligand–Fe(III) complex, providing a high hydroxylation yield. In our experiments, it was clearly shown that benzene hydroxylation was enhanced by the 1:1 catechol–Fe(III) system (yield 0.05%, based on the substrate) rather than by the synthesized 3:1 catechol–Fe(III) complex (yield 0.02%) in the presence of H_2O_2 at pH 4.0. These results suggested that the formation of the 1:1 catechol–Fe(III) complex in solution facilitates the hydroxylation of acetanilide, providing an active oxygen species at the metal complex site [3]. Further studies on the relation between complex formation and generation of active oxygen species in elucidating the hydroxylation mechanism in the present system are in progress.

The addition of hydroxyl radical scavengers such as ethanol (50 m*M*) and formic acid (50 m*M*) to the 3,5-di-t-butylcatechol–Fe(III)–H_2O_2 system resulted in partial inhibition of acetanilide hydroxylation (50 and 64%, respectively). Therefore, it is possible that a hydroxyl radical generated by the system takes part in the hydroxylation of acetanilide.

It is also interesting to note that the stepwise introduction of t-butyl groups into the catechol caused an increase in *p*-hydroxylation and a decrease in *o*-hydroxylation as shown in Table I. In acetanilide hydroxylation catalyzed by rat liver microsomes,

*Author to whom correspondence should be addressed.

TABLE I. Hydroxylation of Acetanilide by Model Systems.

System	Yield[a] (%)	Product Ratio		
		p-AAP :	*m*-AAP :	*o*-AAP
Catechol–Fe(III)–H_2O_2	1.7 ± 0.5	20	5	75
4-t-Butylcatechol–Fe(III)–H_2O_2	3.5 ± 1.0	27	4	69
3,5-di-t-Butylcatechol–Fe(III)–H_2O_2	6.7 ± 1.8	31	5	64
Rat liver microsomes		50	3	47

[a]Based on the substrate concentration. Data are means ± S.D. of the five experiments. [b]See reference [5].

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it was found that the *p*-hydroxylation was preferred to the *o*-hydroxylation, the product ratio (*p*-:*m*-:*o*-AAP) being 50:3:47 [5]. This suggests that further modifications of the catechol molecule in the present system could provide a better chemical model with a reaction of stereochemistry more like that of cytochrome P-450 monooxygenases.

Thus the present substituted Hamilton system, 3,5-di-t-butylcatechol–Fe(III)–H_2O_2, offers not only a simple technique for increasing the hydroxylation of aromatic compounds but also a possible model of biological hydroxylation systems, especially cytochrome P-450 monooxygenases.

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