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# On the Oxygenation of Several Aromatic and Aliphatic Compounds with Aqueous Ferrous Ion-molecular Oxygen[1]

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Hydroxylation was shown to occur readily in the reactions of aromatic compounds such as benzoic acid, nitrobenzene, acetanilide, and phenol with ferrous ion-molecular oxygen in 0.5 M phosphate buffer (pH 6.8) at 40°C for 3 h. The yields of hydroxylated products were higher than those obtained with other reported hydroxylation systems of transition metal-molecular oxygen. The NIH shift was not observed in the title reactions of C(4)-deuterioacetanilide and C(4)-deuteriobenzoic acid. This system was also demonstrated to oxygenate caproic acid to give a mixture of 3-, 4-, and 5-oxocaproic acids in 67% total yield.

**Keywords**——hydroxylation of aromatic compound; ferrous ion; molecular oxygen; benzoic acid; nitrobenzene; acetanilide; phenol; NIH shift; oxygenation of caproic acid; GC-MS of methyl oxocaproate

Numerous chemical model systems for monooxygenase have been proposed, and oxygenation with these systems, consisting of a low-valent transition metal and molecular oxygen, has been studied by many investigators.[2] Udenfriend's system,[3] Fe(II)/ethylenediaminetetraacetic acid (EDTA)/ascorbic acid/$O_2$, is particularly well-known and has been demonstrated to hydroxylate various aromatic compounds.[4] This system and modified systems based on it also hydroxylate alkanes[5] and steroidal compounds such as corticoids[6] and bile acids,[7] though the yields of oxygenated products were low.

In our previous paper of this series, it was demonstrated that adamantane-1-acetic acid and adamantane-1-carboxylic acid were oxygenated quantitatively by the simple model system of ferrous ion-molecular oxygen in 0.5 M phosphate buffer (pH 6.8).[8] These results prompted us to examine the oxygenation of various types of organic compounds with this system. In this report, the reactions of benzoic acid, nitrobenzene, acetanilide, phenol, and caproic acid with this system are described.

## Results and Discussion

### Hydroxylation of Aromatic Compounds

Ferrous sulfate solution was added to a 0.5 M phosphate buffer (pH 6.8) solution of benzoic acid, nitrobenzene, acetanilide, or phenol and the reaction mixture was bubbled through with molecular oxygen at 40°C for 3 h. After usual work-up, the reaction mixture of benzoic acid was methylated with diazomethane and analyzed by gas chromatography (GC). The reaction mixtures of the other aromatic compounds were analyzed by high performance liquid chromatography (HPLC) without derivatization. Although the consumptions of these substrates were shown to exceed 50%, some of their products could not be identified because of failure to extract them from the reaction mixture and/or because of the lack of authentic samples.

A gas chromatogram of the methylated reaction mixture of benzoic acid is shown in Fig. 1. Dihydroxylated products, 2,3-, 2,5-, and 3,4- (or 3,5-) dihydroxybenzoic acids, were identified in addition to the monohydroxylated products, 2-, 3-, and 4-hydroxybenzoic acids. The consumption of benzoic acid was found to be 73%.

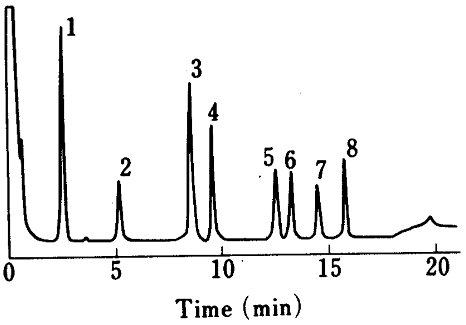

Fig. 1. Gas Chromatogram of the Reaction Mixture of Benzoic Acid

Peaks: (1) benzoic acid, (2) 2-hydroxybenzoic acid, (3) 3-hydroxybenzoic acid, (4) 4-hydroxybenzoic acid, (5) 2,5-dihydroxybenzoic acid, (6) 2,3-dihydroxybenzoic acid, (7) 3,4- or 3,5-dihydroxybenzoic acid.
Conditions: see text.

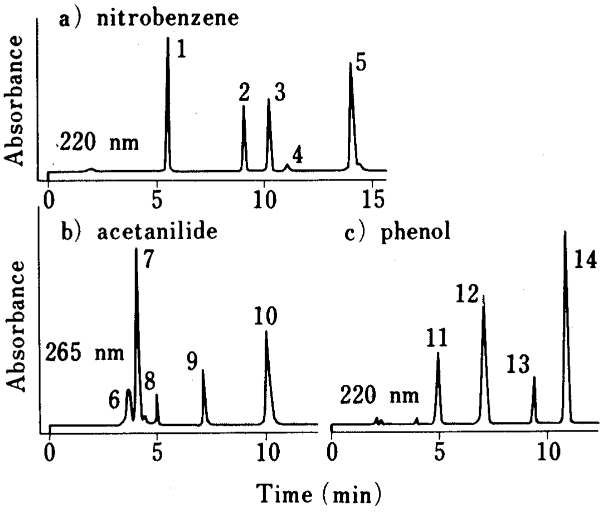

Fig. 2. HPLC Chromatograms of the Reaction Mixtures of Nitrobenzene, Acetanilide, and Phenol

Peaks: (1) unidentified product, (2) 4-nitrophenol, (3) 3-nitrophenol, (4) 2-nitrophenol, (5) nitrobenzene, (6) dihydroxyacetanilide, (7) 4-hydroxyacetanilide, (8) 3-hydroxyacetanilide, (9) 2-hydroxyacetanilide, (10) acetanilide, (11) hydroquinone, (12) catechol, (13) unidentified product, (14) phenol.
Conditions: see text.

Although 77% of nitrobenzene was consumed in the reaction, only about 10% total yield of products could be extracted from the reaction mixture. HPLC of the extracts (Fig. 2a) showed the formation of equal amounts of 3- and 4-nitrophenols (4%), together with a small amount of 2-nitrophenol (1%).

HPLC of the reaction mixtures of acetanilide and phenol are shown in Figs. 2b and 2c, respectively. The consumption of acetanilide was 82% and the products identified were 2-, 3-, and 4-hydroxyacetanilides in a ratio of 5:2:7. The peak at a retention time of about 3 min was indicated to be due to a dihydroxylated product by gas chromatography–mass spectrometric (GC–MS) measurement, though the positions of hydroxylation remained uncertain. In the reaction of phenol, the consumption was 58% and catechol and *p*-hydroquinone were formed in 21 and 7% yields, respectively. No evidence was obtained for the formation of resorcine, *p*-benzoquinone, or pyrogallol.

These results are summarized in Table I. It is noteworthy that the consumptions of these aromatic substrates are considerably higher in this system than in other model systems of transition metal-molecular oxygen previously reported.[4,9] Although the ratio of *o*-, *m*-, and *p*-hydroxylation seems to indicate the electrophilic nature of the attacking species in the title reaction, a more detailed discussion is not possible at present because of the high proportions of unidentified products.

In relation to the enzyme-catalyzed hydroxylation of aromatic substrates involving the NIH shift,[10] hydroxylations with model systems have been carried out on deuterium- or tritium-labelled aromatic compounds. For example, about 10% NIH shift was involved in the reaction of C(4)-tritium labelled acetanilide with peroxytrifluoroacetic acid, which was considered to be a hydroxyl cation-generating system.[11,12] On the other hand, the shift was shown to be less than 1% in the reaction with Udenfriend's system.[11] Determination of the extent of the NIH shift during aromatic hydroxylation would provide further information on the nature of the attacking species involved in the title reaction. Thus, the reaction of C(4)-deuterioacetanilide was examined with this system. GC–MS of the reaction mixture indicated complete loss of deuterium in the 4-hydroxyacetanilide formed. This result is similar to that

TABLE I. Hydroxylations of Aromatic Compounds with the Fe(II)-$O_2$ System

| Compound | Consumption(%) | Yields(%) of hydroxylated products | | | | |
|---|---|---|---|---|---|---|
| | | 2- | 3- | 4- | 2,3- | 2,5- |
| Benzoic acid | 73 | 4 | 14 | 9 | 9 | 5 |
| Nitrobenzene | 77 | 1 | 4 | 4 | — | — |
| Acetanilide | 82 | 5 | 2 | 7 | — | — |
| Phenol | 58 | 21 | — | 7 | — | — |

in Udenfriend's system and is markedly different from that obtained with peroxytrifluoroacetic acid or microsomal oxygenases.[10] No NIH shift was observed in the title reaction of C(4)-deuteriobenzoic acid.

**Oxygenation of Caproic Acid**

Caproic acid (hexanoic acid) was used as a water-soluble aliphatic substrate and its oxygenation was carried out under the same conditions as described above. After usual work-up, the reaction mixture was methylated with diazomethane and analyzed by GC and GC–MS. A gas chromatogram of the methylated reaction mixture is shown in Fig. 3; 67% of the caproic acid was consumed and three oxygenated products were formed. Oxidation of the reaction mixture with Jones's reagent ($CrO_3$–$H_2SO_4$–acetone) produced no change in its chromatogram. Mass spectra of the three products gave a common molecular ion at $m/e$ 144, indicating that these products are isomers of methyl oxocaproate. The positions of the oxo groups may readily be deduced from their MS fragmentation patterns (Fig. 4).[13] Product-1 was concluded to be methyl 3-oxocaproate from the intense ion at $m/e$ 71 (base peak), besides the fragment ions at $m/e$ 43 and 101 resulting from the cleavage of the bond next ($\alpha$) to the oxo group. The structure was also supported by the presence of the ion at $m/e$ 116 produced by 4.5-cleavage ($\beta$ to 3-oxo group) with migration of a hydrogen atom.

The MS of product-2 exhibited ions at $m/e$ 115, 87, and 57 resulting from cleavage $\alpha$ to the 4-oxo group. Thus, product-2 was identified as methyl 4-oxocaproate. Product-3 was concluded to be methyl 5-oxocaproate from the presence of an acylium ion at $m/e$ 43 (base peak) resulting from cleavage $\alpha$ to the 5-oxo group and also the ion at $m/e$ 74 formed by cleavage $\beta$ to the ester carbonyl group with Maclafferty rearrangement. The yields of the three oxygenated products in the reaction of caproic acid are shown in Chart 1.

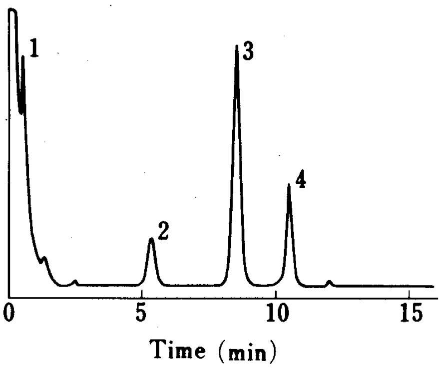

Fig. 3. Gas Chromatogram of the Reaction Mixture of Caproic Acid

Peaks: (1) methyl caproate, (2) methyl 3-oxocaproate, (3) methyl 4-oxocaproate, (4) methyl 5-oxocaproate.
Conditions: see text.

In our previous paper, adamantanes known to be reactive compounds were demonstrated to be oxygenated quantitatively by the system of ferrous ion and molecular oxygen in 0.5 M phosphate buffer (pH 6.8).[8] The present study showed that this simple system also oxygenates some familiar aromatic compounds such as acetanilide, benzoic acid, nitrobenzene, and phenol, and an aliphatic compound, caproic acid, in high yields. Many systems consisting of a transition metal and molecular oxygen have been studied for the hydroxylations of aromatic compounds in the presence or absence of reductant and metal ligand,[4,9] though the yields of hydroxylated products were generally low. The hemin-thiol-molecular oxygen system, which is an excellent chemical model for cytochrome P-450 monooxygenases,[14] also showed poor ability to hydroxylate the substrate. Of these systems, the highest hydroxylation yield was given by the system of Fe(II)-EDTA-molecular oxygen; the maximum yield was 13%.[9b] The yields of oxygenated products were also found to be low in the reactions of aliphatic com-

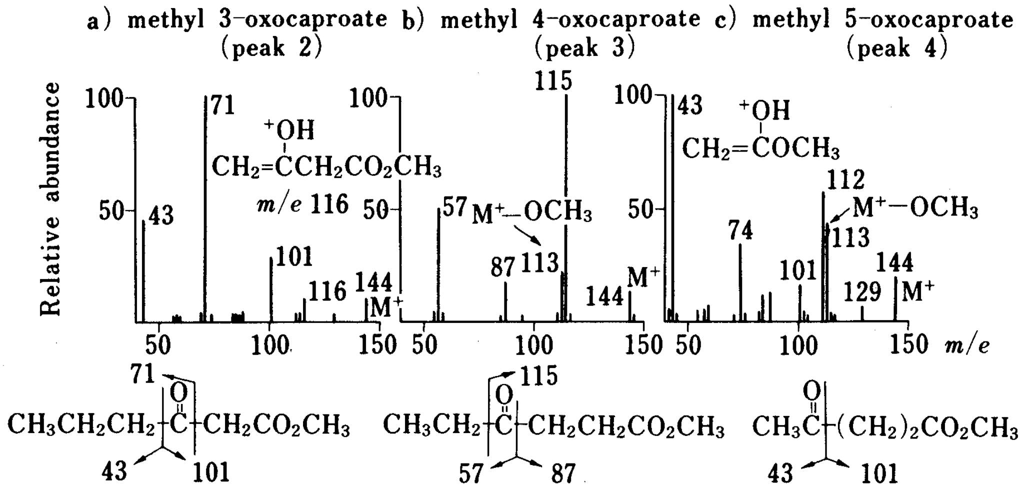

Fig. 4. Mass Spectra of Methyl Oxocaproates

$$CH_3(CH_2)_4COOH \xrightarrow{Fe(II)\text{-}O_2} \begin{array}{c} CH_3CH_2CH_2COCH_2COOH \\ 9\ \% \\ CH_3CH_2COCH_2CH_2COOH \\ 37\ \% \\ CH_3COCH_2CH_2CH_2COOH \\ 21\ \% \end{array}$$

Chart 1

pounds with various systems of transition metal and molecular oxygen.[5] However, the systems could oxygenate aliphatic compounds in high yield under special conditions. For example, Hanotier *et al.* reported that heptane was readily oxygenated to a mixture of heptanones in 80% yield by Co(III)-$O_2$ in acetic acid containing a strong acid such as sulfuric acid or trichloroacetic acid.[15] Recently, by using $Fe(ClO_4)_2$-$O_2$ in acetic acid at 120°C, Patin *et al.* found that cholestane-3$\alpha$-yl-xanthate was, as a result of the template effect of the xanthate group, oxygenated stereoselectively to the C(1) $\alpha$-acetate in 45% yield.[16] In this study, the simple system consisting of ferrous ion and molecular oxygen was also demonstrated to oxygenate unactivated carbon atoms in an aliphatic compound. It is of great interest that this simple system can oxygenate various types of organic compounds under mild conditions. The mechanism of the title reaction, however, remains uncertain and is now under investigation.

## Experimental

**Materials**———Aromatic compounds, the authentic hydroxylated products, and caproic acid were purchased commercially. Phenyl diazomethane,[17] 4-deuterioacetanilide (92% deuterium content),[18] and 4-deuteriobenzoic acid (93% deuterium content)[18] were prepared by the cited methods.

**Methods**———Gas chromatographic data were taken on a Shimadzu GC-4BM or GC-4CM gas chromatograph equipped with a hydrogen flame ionization detector and a 2 m glass column packed with 5% OV-17, 5% XF-1105, or 1.5% SE-30. HPLC was carried out at 25°C with a Shimadzu LC-2 chromatograph, a variable wavelength ultraviolet (UV) detector (SPD-2A, Shimadzu), and a stainless steel column (25 cm×4.6 mm i.d.) packed with Zorbax ODS (5 $\mu$m, Shimadzu). GC–MS was carried out on a JEOL JMS-D-300 instrument using a 10% SE-30 column (2 m×3 mm i.d.) or a 5% XF-1105 column (1 m×3 mm i.d.). The mass spectra were recorded at an electron energy of 70 eV, a trap current of 60 $\mu$A, an acceleration voltage of 3.0 kV, an ion source temperature of 150°C, and a molecular separator temperature of 250°C; helium was used as a carrier gas at a pressure of 0.6 kg/cm$^2$.

**General Procedure for the Title Reaction**———A suspension of the substrate (0.1 mmol) in 0.05 ml of 2 M NaOH was dissolved in 100 ml of 0.5 M phosphate buffer (pH 6.8) and then mixed with ferrous ion solution (4.3

mmol of $FeSO_4 \cdot 7H_2O$ in 5 ml of water). Oxygen was bubbled through the mixture for 3 h at 40°C under continuous stirring. A portion (5 ml) of the reaction mixture was adjusted to pH 2 with 10% HCl. The resultant homogeneous solution was saturated with NaCl and extracted with AcOEt (2 ml×4). The organic layer was washed with aq. NaCl (3 ml), and concentrated to dryness under an $N_2$ stream. The residue thus obtained was analyzed by GLC or HPLC as described below.

**Determination of Substrate Consumption and Product Yield**——a) Benzoic Acid: The residue thus obtained was dissolved in 2 ml of MeOH–$Et_2O$ (1:3) containing 1-adamantanol as an internal standard, methylated with diazomethane, and subjected to GLC on a 1.5% SE-30 column. The column was operated isothermally at 100°C for 6 min after the injection, then programmed to 180°C at a rate of 6°C/min. $N_2$ was used as a carrier gas at a flow rate of 50 ml/min.

b) Nitrobenzene, Acetanilide, and Phenol: The residue obtained from each of the reaction mixtures was dissolved in 2 ml of MeOH containing an internal standard (phenol for nitrobenzene, catechol for acetanilide, and 2-hydroxyacetanilide for phenol) and subjected to HPLC. MeOH–$H_2O$ (1:1 for nitrobenzene, 2:3 for acetanilide and phenol) was employed as a mobile phase at a flow rate of 2 ml/min and the effluent was monitored by UV absorption measurement at 220 nm for nitrobenzene and phenol, and at 265 nm for acetanilide.

Each peak in the chromatograms (Figs. 1, 2a, b, and c) was identified by comparison of the retention time with that of an authentic standard. Peak-6 in the chromatogram (Fig. 2b) was indicated to be due to dihydroxyacetanilide by the presence of the molecular ion at *m/e* 195 in the mass spectrum (GC–MS, column of 2 m×3 mm i.d. with 10% SE-30, column temperature of 150°C) of the methylated reaction mixture. The recovery rates from the reaction mixtures were 61, 72, and 87% for nitrobenzene, catechol, and hydroquinone, respectively. The racovery rates of the other aromatic substrates and their hydroxylated products (Figs. 1, 2b, and 2c) were demonstrated to be in the range of 90—100% by recovery tests. The substrate consumptions and product yields were calculated by comparison of the peak heights with those of authentic standards and correcting the results for the recovery rates.

c) Caproic Acid: A portion of the residue obtained from the reaction of caproic acid was dissolved in *n*-pentane containing caprylic acid as an internal standard, benzylated with phenyl diazomethane, and subjected to GLC (column, 2 m×3 mm i.d. with 5% OV-17; temperature, 180°C) for determination of the substrate consumption. The recovery of caproic acid was found to be 90%. Another portion of the residue was methylated with diazomethane and subjected to GLC (column, 2 m×3 mm i.d.; 5% XF-1105; 110°C), and GC–MS (column, 1 m×3 mm i.d.; 5% XF-1105; 80°C). The yields of the oxygenated products were determined from the gas chromatogram (Fig. 3) without correction for relative sensitivities to the hydrogen flame ionization detector (FID).

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