Relation between Electronic Structure and Hydroxylation of Aromatic Compounds by Microorganisms

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The π-electron distribution of various aromatic compounds has been calculated by a molecular orbital method.

The reaction of hydroxylation was assumed to be radical type.
Relation between electronic structures and mono-hydroxylation of aromatic compounds by microorganisms was investigated.

A distinct parallelism was ruled out between the electronic structure and hydroxylation of aromatic compounds.

Hydroxylation of aromatic compounds occurred where the superdelocalizability Sr (R) showed large value.

There are many reports which are concerned with microbial hydroxylation of aromatic compounds. These reports mainly demonstrated the hydroxylated products and enzymes which catalyzed the hydroxylation. But there are no reports which describe the relation between electronic structure and microbial hydroxylation of aromatic compounds.

This paper refers to relations between π-electron structure and the position where mono-hydroxylation occurs by microbial reactions for various aromatic substrates.

THEORETICAL INDEX FOR ACTIVITY

We do not describe the details of frontier electron theory in this paper but briefly explain the theoretical index as a measure of chemical reactivity of conjugated molecules.

Frontier electron density has been demonstrated to be a good index for debating intramolecularly the chemical reactivity of aromatic compounds. A position which has the largest frontier electron density is most susceptible to attack, not only in the case of substitution but also in the case of addition. Frontier electrons are defined as the two electrons occupying the highest molecular orbital in the ground state in the case of reaction with an electrophilic reagent. In the case of reaction with a nucleophilic reagent, the frontier orbital is the lowest vacant orbital of the ground state; and, in the case of reaction with a radical reagent, both the two orbitals mentioned above.

Superdelocalizability was defined according to the type of reaction as

(a) for an electrophilic reaction

$$Sr(E) = 2 \sum_{j=1}^{m} \frac{Cr(j)}{\lambda_j}$$

(b) for a radical reaction

$$Sr(R) = \sum_{j=1}^{m} \frac{Cr(j)}{\lambda_j} + \sum_{j=m+1}^{N} \frac{Cr(j)}{(-\lambda_j)}$$

(c) for a nucleophilic reaction

$$Sr(N) = 2 \sum_{j=m+1}^{N} \frac{Cr(j)}{(-\lambda_j)}$$

Where N is the total number of π-orbitals in the molecule; the occupied levels are denoted by 1, 2, ... m and the unoccupied levels by m+1, m+2, ... N, Cr(j) is the coefficient of rth atomic π orbital in the jth molecular orbitals.
tal, and $\lambda_j$ is the coefficient of the resonance integral, when the energy of a molecular orbital is expressed in the form $a + \lambda_j \beta$. $a$ and $\beta$ are the coulomb and the resonance integral of a carbon atom and C–C bond in benzene, respectively. The Sr value has been demonstrated to be a good index for debating intramolecular and intermolecular reactivity. The position which shows the largest Sr value is most susceptible to attack.

PARAMETER USED IN CALCULATION

The calculation of Sr is carried out by using the simple LCAO–MO (linear-combination-of-atomic-orbitals molecular orbital) treatment, solving the secular equation. The coulomb integral of the two equivalent oxygen atoms in the –COO groups is taken as $a + 2\beta$. The coulomb integral of the substituent $X$, that of the carbon atom attached to $X$, and the resonance integral between that carbon atom and $X$ are written as $a + a_x \beta$, $a + a_r \beta$, and $l \beta$, respectively. The numerical values of $a_x$, $a_r$, and $l \beta$ adopted by us are shown in Table I.

TABLE I. PARAMETERS USED IN THE CALCULATION

<table>
<thead>
<tr>
<th>Substituent $X$</th>
<th>$a_x$</th>
<th>$a_r$</th>
<th>$l$</th>
</tr>
</thead>
<tbody>
<tr>
<td>=O</td>
<td>2</td>
<td>0.2</td>
<td>1.4</td>
</tr>
<tr>
<td>=O–</td>
<td>2</td>
<td>0.2</td>
<td>0.6</td>
</tr>
<tr>
<td>=OH</td>
<td>0.6</td>
<td>0</td>
<td>0.7</td>
</tr>
</tbody>
</table>

C–C ≡ H$_3$

0.7$\beta$ 2.5$\beta$

$a - 0.1\beta$ $a - 0.5\beta$

RESULTS AND DISCUSSION

There are many studies on the hydroxylation of aromatic compounds. Some of these works are as follows.

Evans$^1$ reported the hydroxylation of phenol to catechol and benzoic acid to $p$-hydroxybenzoic acid as shown in Tables II and IV. Voets$^2$ recognized the hydroxylation of benzoic acid to salicylic acid as listed in Table IV. Omori and Yamada$^3$ found out the formation of 3-methyl salicylic acid from $m$-toluic acid as presented in Table III. Mason et al.$^4$ demonstrated the hydroxylation of 3,4-dimethylphenol to 3,4-dimethylcatechol by phenolase complex as shown in Table V. Yano and Arima$^5$ reported the formation of protocatechuic acid and gentisic acid from $m$-hydroxybenzoic acid as presented in Table VI.

The values of superdelocalizability of these substrates mentioned above were calculated and the results of the calculation of superdelocalizability are indicated in Tables II~VI.

It was assumed that the mono-hydroxylation of aromatic compounds was the type of radical reaction, since it is generally considered that molecular oxygen takes part in the mono-hydroxylation of aromatic compound.

When phenol or $m$-toluic acid was used as substrate, microbial hydroxylation of unoccupied benzene nucleus occurred where the largest value of Sr($R$) was found as shown in Tables II and III. Thus, the prediction by means of the Sr($R$) value agreed with experimental results.

TABLE II. HYDROXYLATION OF PHENOL TO CATECHOL

<table>
<thead>
<tr>
<th>Substituent $X$</th>
<th>Sr($R$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>=O</td>
<td>0.1778×10</td>
</tr>
<tr>
<td>=O–</td>
<td>0.7732</td>
</tr>
<tr>
<td>=OH</td>
<td>0.9643</td>
</tr>
</tbody>
</table>

TABLE III. HYDROXYLATION OF $m$-TOLUIC ACID TO 3-METHYLSALICYLIC ACID

<table>
<thead>
<tr>
<th>Substituent $X$</th>
<th>Sr($R$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>=O</td>
<td>0.4374</td>
</tr>
<tr>
<td>=O–</td>
<td>0.4024</td>
</tr>
<tr>
<td>=OH</td>
<td>0.8150</td>
</tr>
<tr>
<td>=O</td>
<td>0.9416</td>
</tr>
<tr>
<td>=O–</td>
<td>0.8283</td>
</tr>
<tr>
<td>=OH</td>
<td>0.9593</td>
</tr>
<tr>
<td>=O</td>
<td>0.7594</td>
</tr>
<tr>
<td>=O–</td>
<td>0.9849</td>
</tr>
<tr>
<td>=OH</td>
<td>1.325×10</td>
</tr>
</tbody>
</table>

In other cases such as benzoic acid, 3,4-dimethylphenol and $m$-hydroxybenzoic acid, hydroxylation occurred at a position which shows the largest or near to the largest Sr($R$) value as presented in Tables IV~VI. Con-
TABLE IV. HYDROXYLATION OF BENZOIC ACID TO SALICYLIC ACID AND p-HYDROXYBENZOIC ACID

\[
\begin{array}{c}
\text{Sr}(R) \\
1) 0.6258 \\
2) 0.9848 \\
3) 0.1325 \times 10 \\
4) 0.7598 \\
5,9) 0.9525 \\
6,8) 0.8290 \\
7) 0.9315
\end{array}
\]

TABLE V. HYDROXYLATION OF 3,4-DIMETHYLPHENOL TO DIMETHYLCATECHOL

\[
\begin{array}{c}
\text{Sr}(R) \\
1) 0.4459 \\
2) 0.4019 \\
3) 0.9368 \\
4) 0.8387 \\
5) 0.9681 \\
6) 0.7771 \\
7) 0.1772 \times 10 \\
8) 0.9745 \\
9) 0.8252 \\
10) 0.4024 \\
11) 0.4382
\end{array}
\]

TABLE VI. HYDROXYLATION OF m-HYDROXYBENZOIC ACID TO PROTOCATECHUIC OR GENTISIC ACID

\[
\begin{array}{c}
\text{Sr}(R) \\
1) 0.7536 \\
2) 0.1091 \times 10 \\
3) 0.2503 \times 10 \\
4) 0.7544 \\
5) 0.1082 \times 10 \\
6) 0.8245 \\
7) 0.1079 \times 10 \\
8) 0.7699 \\
9) 0.1779 \times 10 \\
10) 0.1116 \times 10
\end{array}
\]

Considering a possibility of steric hindrance to hydroxylation at a position sandwiched by two ortho substituents, these data could be taken to show a parallelism of \( \text{Sr}(R) \) value with experimental results of hydroxylation of aromatic compounds.

Our overall results showed good correlation between the position of hydroxylation of benzene nucleus and the value of \( \text{Sr}(R) \). It is necessary to demonstrate experimentally as to whether the microbial hydroxylation actually occurs via a radical mechanism or not. It is also interesting to find out exceptions from the above rule, since the enzyme which catalyzes such exceptional cases seems to be stereo-electronically specific.

Acknowledgement. Grateful acknowledgement is made to Dr. Akira Imamura for the calculation of the values of superdelocalizability \( \text{Sr}(R) \) and for his appropriate advice.

REFERENCES