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Quantum Chemical Study on the Cytochrome P-450-Catalyzed Hydroxylation of 1,2,3,4-Tetrahydronaphthalene and Indane

EIJI YUKAWA, KEIKI KOUNO, YUKIO ONO, and YO UEDA*

Faculty of Pharmaceutical Sciences, Kyushu University Maedashi 3-1-1, Higashi-ku, Fukuoka 812, Japan

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A quantum chemical method for prediction of the specific atom of 1,2,3,4-tetrahydronaphthalene or indane at which hydroxylation will occur most easily in liver microsomes is presented.

The interaction energy changes of a binary system composed of the hydrocarbon and O_2 caused by changes of the mutual spatial arrangement between the hydrocarbon and O_2 were calculated by the CNDO/2 method.

The theoretical predictions were found to coincide very well with the reported experimental results.

Keywords——hydroxylation in liver microsomes; cytochrome P-450; CNDO/2 method; 1,2,3,4-tetrahydronaphthalene; indane; interaction energy

It was found in our previous paper[1] that the ease of cytochrome P-450-catalyzed epoxidation of aromatic hydrocarbon at its specific bond can be explained satisfactorily by the spatial arrangement of the molecule in the cytochrome P-450-O_2-substrate complex. We considered that the spatial arrangement of a substrate molecule in the above-mentioned complex is one of the important factors which determine the specific moiety of the molecule at which the monooxygenase reaction takes place. Since other factors, such as the role of amino acid residues of cytochrome P-450 in the formation of the enzyme-substrate complex, are difficult to include in the quantum chemical calculation, only the spatial arrangement of a substrate molecule was used in looking for the moiety of the molecule at which the oxidation reaction is expected to take place most easily. Moreover, the spatial arrangement of a substrate molecule, whose variation was the subject of our consideration, was taken to be that in a simple model system composed of only O_2 and a substrate molecule. Nevertheless, satisfactory results were obtained in all of the cases examined in our previous work. Thus, we extended our work to the cases of other types of monooxygenase reactions of drug molecules in liver microsomes. In the present investigation, we took up the case of cytochrome P-450-catalyzed hydroxylation of a drug molecule at a specific carbon atom of the alicyclic ring of the molecule.

1,2,3,4-Tetrahydronaphthalene and indane, which belong to the group of substrates showing Type I difference spectra, were selected as examples of hydrocarbons having an alicyclic ring. 1,2,3,4-Tetrahydronaphthalene was reported to be oxidized mainly at its $C_{(1)}$ atom and to a lesser extent at its $C_{(2)}$ atom to give monohydroxy derivatives in the animal body.[2] Indane was reported to be converted to indan-1-ol in liver microsomes.[3] These experimental results mean that the cytochrome P-450-catalyzed oxidation reaction took place preferentially at specific carbon atoms of the alicyclic rings of two compounds.

As in the previous work,[1] it was presumed that the oxidation reaction would take place most easily at the carbon atom located in the closest proximity to O_2 in the ternary complex. The electronic state of the oxygen in the ternary cytochrome P-450-O_2-substrate complex, which has not yet been clarified, was assumed to be the singlet O_2 state in the present investigation, by analogy with the states of oxygen in the iron (II) dioxygen complexes of porphyrins reported in the literature. The electronic states of oxygen in the iron (II) dioxygen complexes of the "picket fence porphyrin"[4] and of the ammine-porphyrin[5] were both considered to be the singlet O_2 states on the basis of X-ray structural characterization and infrared absorption spectroscopy[4] and the result of *ab initio* calculation,[5] respectively. The reaction between a singlet substrate molecule and the triplet oxygen molecule is a spin-forbidden process, so that substantial activation energy would be required. This "symmetry barrier" may be overcome by forming singlet complexes between transition metals and oxygen.[6] For the reasons mentioned in the previous paper,[1] a simple model system composed of only O_2 and a substrate molecule (which will be called the binary system) was used in the calculation of total energies of binary systems.

The results of calculation by the CNDO/2 method are known to have inherent shortcomings as a result of approximations involved in the method, as mentioned in the previous paper. Nevertheless, this method has been reported to give fairly reliable, though qualitative, results in molecular orbital calculations for moderately large molecules and systems composed of a few molecules interacting with each other. For example, the ground state molecular orbitals of the free base porphine calculated by the CNDO/2 method[7] were found to be in good overall agreement with the level pattern calculated by *ab initio* calculation, apart from some differences of ordering.[8] Umeyama *et al.*[9] reported that the potential curve calculated by the CNDO/2 method for a hydrolysis by α-chymotrypsin reproduces the experimental curve well and the relative magnitudes of all steps in the reaction path explain the experimentally obtained activation energies satisfactorily. The catalytic reaction mechanism of α-chymotrypsin was also studied by Beppu *et al.*,[10] and the mechanism obtained by CNDO/2 calculation was found to agree well with the mechanism proposed experimentally by other researchers. Judging from these findings, we considered that the CNDO/2 method was applicable in the present investigation.

Because of the unavoidable shortcomings caused by the use of the CNDO/2 method (the optimized intermolecular distances are unreliable), it was impossible to determined the precise mutual spatial arrangements of components of the binary system. The only thing we can do is to estimate the degree of steric hindrance which may exist between the substrate molecule and the heme plane. However, this problem was not considered to be too serious in the present investigation, as in the previous investigation, since our aim was only to identify the specific atom at which the oxidation reaction should take place most easily.

Results and Discussion

The Method of Investigation

Both 1,2,3,4-tetrahydronaphthalene and indane have two kinds of carbon atoms, $C_{(1)}$ and $C_{(2)}$, in their alicyclic rings. Therefore, O_2 was brought nearer to these atoms, and the accompanying change of the interaction energy, which is defined as the energy difference between the total energy of the binary system itself and the sum of the total energies of the two component molecules of the binary system, was calculated by the CNDO/2 method. It can again be considered that the smaller the interaction energy (ΔE) is, the more stable the binary system is. For convenience in the molecular orbital calculation, the approach of O_2 to each one of the two kinds of carbon atoms was devided into three steps, A, B and C.

As the A step, O_2 was brought nearer to either the $C_{(1)}$ or the $C_{(2)}$ atom along one of three straight lines (Fig. 1). In this figure, $C_{(j)}$ denotes either the $C_{(1)}$ or the $C_{(2)}$ atom of both 1,2,3,4-tetrahydronaphthalene and indane, $C_{(i)}$ denotes either the $C_{(8a)}$ or the $C_{(1)}$ atom of 1,2,3,4-tetrahydronaphthalene and either the $C_{(7a)}$ or the $C_{(1)}$ atom of indane, $C_{(k)}$ denotes either the $C_{(2)}$ or the $C_{(3)}$ atom of both compounds, $H_{(a)}$ and $H_{(e)}$ denote the H atoms of the quasi-axial C–H bond and of the quasi-equatorial C–H bond, respectively. Line I is defined as the one located on the plane of three atoms, $H_{(a)}$, $C_{(j)}$ and $H_{(e)}$, that passes through three atoms, $O_{(2)}$, $O_{(1)}$ and $C_{(j)}$, and bisects the $H_{(a)}$–$C_{(j)}$–$H_{(e)}$ angle. Lines II and III are defined

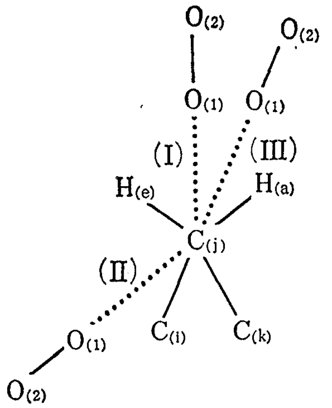

Fig. 1. The Mode of Approach of O_2 to a C Atom (the A Step)

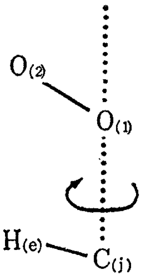

Fig. 2. The Mode of Approach of O_2 to a C Atom (the B Step)

as those which pass through four atoms, $O_{(2)}$, $O_{(1)}$, $C_{(j)}$ and $H_{(a)}$, and $O_{(2)}$, $O_{(1)}$, $C_{(j)}$ and $C_{(i)}$, respectively. The heme plane is not shown in this figure, but the $O_{(2)}$ atom must be considered to combine directly with the Fe atom of the heme.

As the B step, the $C_{(j)}$–$O_{(1)}$–$O_{(2)}$ angle was changed to 109.47°, and the $O_{(1)}$–$O_{(2)}$ bond was rotated about the $C_{(j)}$–$O_{(1)}$ axis counterclockwise from the viewpoint at the $C_{(j)}$ atom, while the distance between the $C_{(j)}$ and the $O_{(1)}$ atoms found to be optimum in the A step was kept invariable (Fig. 2). This rotation angle, θ, was defined as 0° when the $O_{(2)}$ atom is located on the plane of $O_{(1)}$, $C_{(j)}$ and $H_{(e)}$ atoms on the same side as the $H_{(e)}$ atom with respect to the dotted line shown in Fig. 2; this line corresponds to some of the three lines defined before. The change of the $C_{(j)}$–$O_{(1)}$–$O_{(2)}$ angle was based on the facts that the C–O–O angle in dimethyl peroxide was reported to be $105 \pm 3°$[11] and that the O atom with connectivity 2 having bent geometry is usually treated as having a bond angle of 109.47° in the CNDO/2 method.[12]

As the C step, the distance between the $C_{(j)}$ and the $O_{(1)}$ atoms was adjusted again to make the total energy of the binary system minimum, while the angle (θ) found to be optimum in the B step was kept invariable.

Interaction energies of all mutual spatial arrangements of the binary system optimized by the above-mentioned method for the approaches of O_2 to both the $C_{(1)}$ and the $C_{(2)}$ atoms were compared, and the arrangement having the smallest interaction energy was selected as the most stable mutual spatial arrangement of the binary system.

Lastly, the spatial relationship between the heme plane and 1,2,3,4-tetrahydronaphthalene or indane was examined geometrically. In this examination, the Fe–$O_{(2)}$ bond length and the Fe–$O_{(2)}$–$O_{(1)}$ angle were assumed to be 1.75 Å and 135° from the literature,[4] and the intermolecular distance ($r_{C\text{-}O}$) between the substrate molecule and O_2 of the binary system having the most stable mutual spatial arrangement was used, even though this distance must be considerably shorter than that in the ternary complex. If it can be concluded that O_2 can approach the substrate molecule without suffering steric hindrance by this examination, such an approach should actually be possible in liver microsomes.

When the hydroxylation is going to take place at a carbon atom of a hydrocarbon, the $O_{(1)}$–$O_{(2)}$ bond is expected to become weaker than the same bond in the free state. The resonance energy of a bond is known to correlate closely with the bond energy.[13] Therefore, the resonance energy change of the $O_{(1)}$–$O_{(2)}$ bond ($\Delta E^{R}_{O\text{-}O}$) caused by te approach of O_2 to the hydrocarbon was calculated.

1,2,3,4-Tetrahydronaphthalene

First, O_2 was brought nearer to the $C_{(1)}$ atom of 1,2,3,4-tetrahydronaphthalene along three different lines, and the mode of approach was selected to form the most stable spatial arrangement of the binary system composed of the compound and O_2.

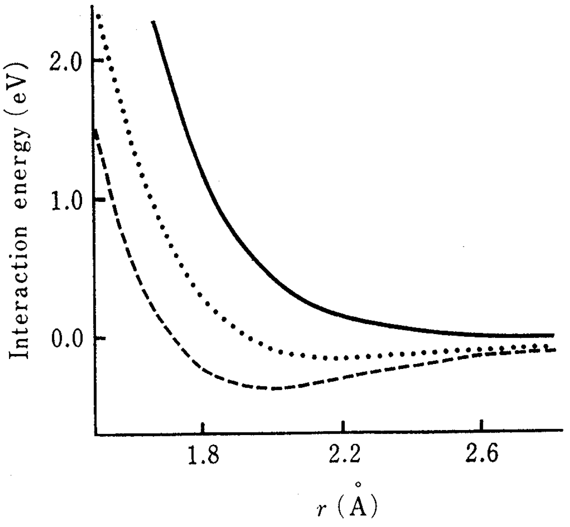

Fig. 3. Interaction Energy Changes due to the A Step of Approach of O_2 to the $C_{(1)}$ Atom of 1,2,3,4-Tetrahydronaphthalene

——: approach along line I,
----: approach along line II,
……: approach along line III.

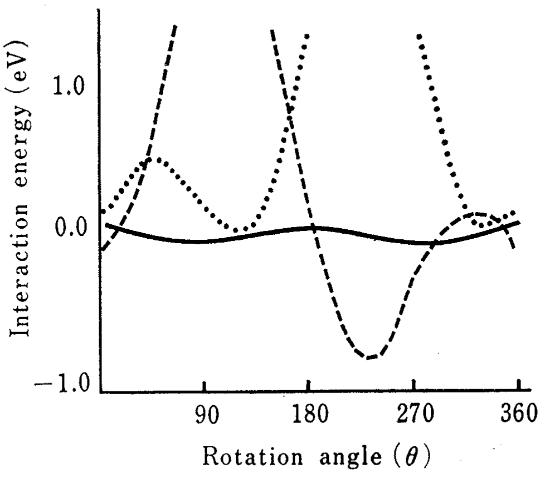

Fig. 4. Interaction Energy Changes due to the B Step of Approach of O_2 to the $C_{(1)}$ Atom of 1,2,3,4-Tetrahydronaphthalene

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----: approach along line II,
……: approach along line III.

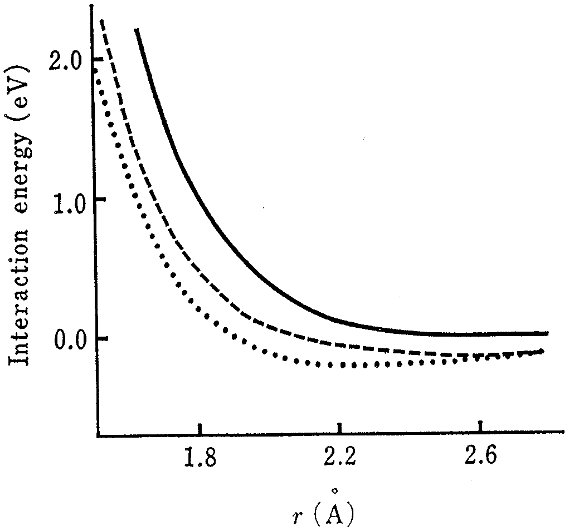

Fig. 5. Interaction Energy Changes due to the A Step of Approach of O_2 to the $C_{(2)}$ Atom of 1,2,3,4-Tetrahydronaphthalene

——: approach along line I,
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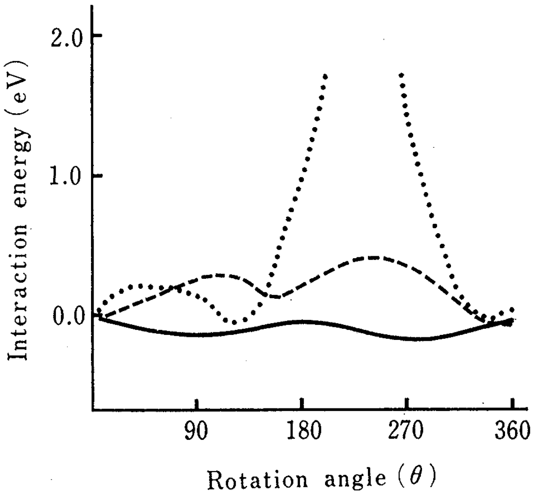

Fig. 6. Interaction Energy Changes due to the B Step of Approach of O_2 to the $C_{(2)}$ Atom of 1,2,3,4-Tetrahydronaphthalene

——: approach along line I,
----: approach along line II,
……: approach along line III.

Fig. 3 shows the interaction energy changes of the binary system caused by the A steps of approach. The curve showing the interaction energy change caused by the approach along line I had an inflection at a distance of 2.2 Å, so this distance was decided to be optimum. In the cases of the approaches along lines II and III, 2.0 Å and 2.2 Å were found to be optimum distances, respectively.

Fig. 4 shows the interaction energy changes of the binary system caused by the B steps of approach. In the case of the approach along line I, the interaction energy did not vary much with change of θ, but since this energy was calculated to be smallest at the angle of 90°, 90° was decided to be optimum. In the case of the approach along line II, 240° was found to be optimum. In the case of the approach along line III, the interaction energies calculated at angles of 120° and 330° were not very different, but the energy calculated at the former angle was smaller than that calculated at the latter angle, so 120° was decided to be optimum.

The results of the C steps of approach indicated that the binary system had the smallest interaction energy, −1.95 eV, when O_2 was brought nearer to the $C_{(1)}$ atom along line II until the distance r_{C-O} was 1.65 Å. The distance of 1.65 Å is considered to be unacceptable as the intermolecular distance between the substrate molecule and O_2 in the ternary complex. However, this mode of approach of O_2 to the substrate molecule is expected to be reasonably reliable, for the reasons mentioned in the previous paper.

In order to estimate the degree of steric hindrance between the substrate molecule and the heme plane, their mutual spatial arrangement was examined geometrically. In this examination, the value of 1.65 Å was used. The result indicated that O_2 can approach the $C_{(1)}$ atom without suffering steric hindrance.

Next, O_2 was brought nearer to the $C_{(2)}$ atom along three different lines.

Fig. 5 shows the interaction energy changes of the binary system caused by the A steps of approach. From the results, distances of 2.2 Å ,2.6 Å and 2.2 Å were decided to be optimum in the approaches along lines I, II and III, respectively.

Fig. 6 shows the interaction energy changes of the binary system caused by the B steps of approach. In the case of the approach along line I, the binary system having a rotation angle of either 90° or 270° was found to be most stable. In the cases of the approaches along lines II and III, 0° and 120° were found to be optimum, respectively.

The results of the C steps of approach indicated that the binary system had the smallest interaction energy, −0.30 eV, when O_2 was brought nearer to the $C_{(2)}$ atom along the line I until the distance r_{C-O} was 1.8 Å, whether the value of θ is 90° or 270°.

The results of geometrical examination, in which the value of r_{C-O} of 1.8 Å was used, indicated that O_2 can approach the $C_{(2)}$ atom without suffering steric hindrance.

Based on a comparison of the magnitudes of interaction energies of the two most stable states of the binary system, the oxidation reaction of 1,2,3,4-tetrahydronaphthalene is pre-

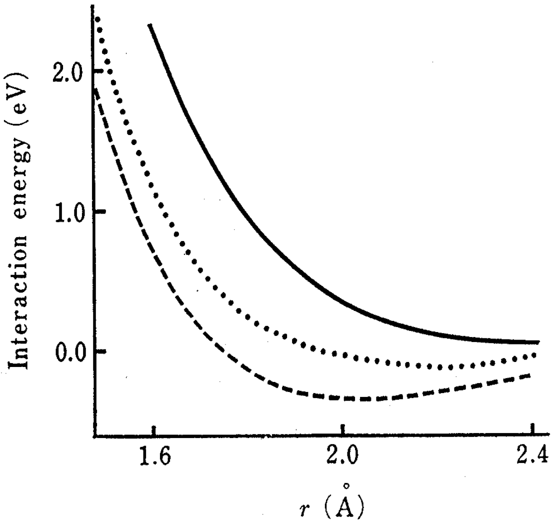

Fig. 7. Interaction Energy Changes due to the A Step of Approach of O_2 to the $C_{(1)}$ Atom of Indane

——: approach along line I,
----: approach along line II,
······: approach along line III.

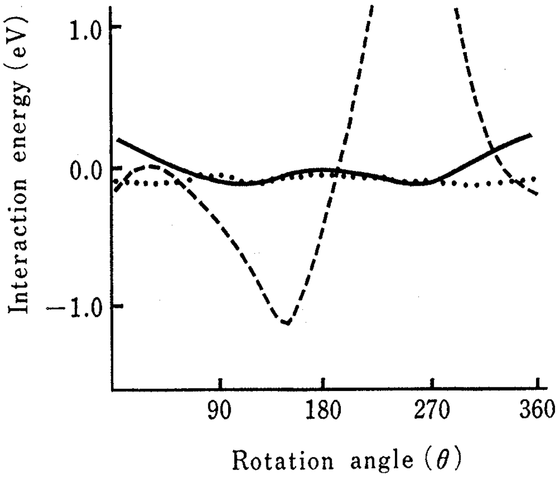

Fig. 8. Interaction Energy Changes due to the B Step of Approach of O_2 to the $C_{(1)}$ Atom of Indane

——: approach along line I,
----: approach along line II,
······: approach along line III.

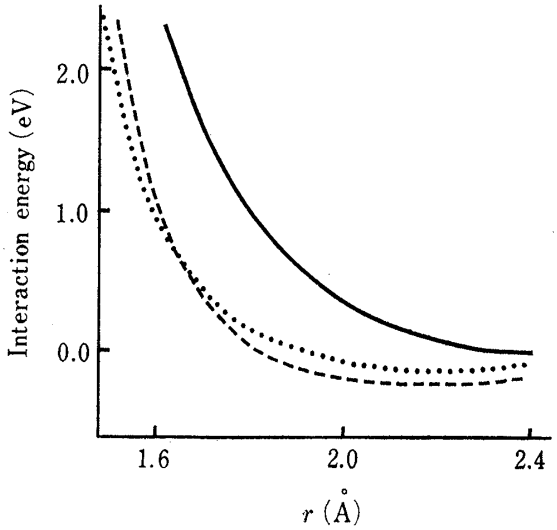

Fig. 9. Interaction Energy Changes due to the A Step of Approach of O_2 to the $C_{(2)}$ Atom of Indane

——: approach along line I,
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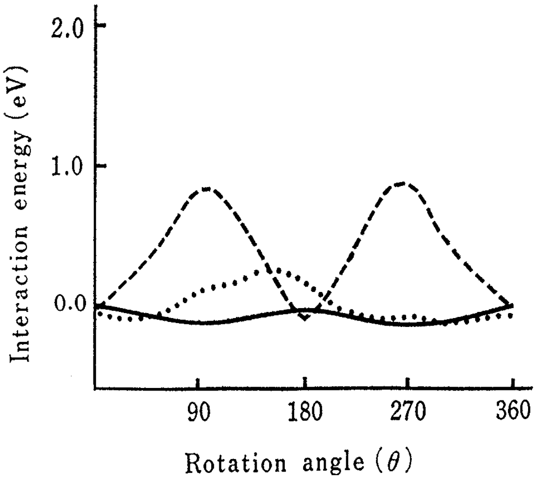

Fig. 10. Interaction Energy Changes due to the B Step of Approach of O_2 to the $C_{(2)}$ Atom of Indane

——: approach along line I,
----: approach along line II,
······: approach along line III.

dicted to take place preferentially at its $C_{(1)}$ atom. This prediction coincides very well with the experimental results.

The resonance energy changes, $\Delta E^{R}_{O\text{-}O}$, were found to be 7.51 and 2.89 eV when O_2 was brought nearer to the $C_{(1)}$ and the $C_{(2)}$ atom, respectively. This result provides further support for the hydroxylation of 1,2,3,4-tetrahydronaphthalene at its $C_{(1)}$ atom in preference to its $C_{(2)}$ atom.

Indane

First, O_2 was brought nearer to the $C_{(1)}$ atom of indane along three different lines, and the mode of approach was selected to form the most stable spatial arrangement of the binary system composed of indane and O_2.

Fig. 7 shows the interaction energy changes of the binary system caused by the A steps of approach. From the results, distances of 2.2 Å, 2.0 Å and 2.2 Å were decided to be optimum in the cases of the approaches along lines I, II and III, respectively.

Fig. 8 shows the interaction energy changes of the binary system caused by the B steps of approach. In the case of the approach along line I, the stability of the binary system having a rotation angle of 120° was found to be not very different from that having an angle of 240°, but 120° was found to be optimum. In the case of the approach along line II, 150° was found to be optimum. In the case of the approach along line III, the interaction energy did not vary much with change of θ, but 300° was found to be optimum.

The results of the C steps of approach indicated that the binary system had the smallest interaction energy, −2.10 eV, when O_2 was brought nearer to the $C_{(1)}$ atom along the line II until the distance $r_{C\text{-}O}$ was 1.7 Å.

The results of geometrical examination, in which the value of $r_{C\text{-}O}$ of 1.7 Å was used, indicated that O_2 can approach the $C_{(1)}$ atom without suffering steric hindrance.

Next, O_2 was brought nearer to the $C_{(2)}$ atom along three different lines.

Fig. 9 shows the interaction energy changes of the binary system caused by the A steps of approach. In the case of the approach along line I, the curve had an inflection at about 2.2 Å, so this distance was decided to be optimum. In the cases of the approaches along lines II and III, 2.2 Å was found to be optimum.

Fig. 10 shows the interaction energy changes caused by the B steps of approach. In the case of the approach along line I, the binary system was found to be most stable when the rotation angle is either 90° or 270°. In the cases of the approaches along lines II and III, 180° and 300° were found to be optimum, respectively.

The results of the C steps of approach indicated that the binary system has the smallest interaction energy, —0.34 eV, when O_2 was brought nearer to the $C_{(2)}$ atom along the line I until the distance r_{C-O} was 1.8 Å, whether the value of θ is 90° or 270°.

The result of the geometrical examination, in which the value of r_{C-O} of 1.8 Å was used, indicated that O_2 can approach the $C_{(2)}$ atom without suffering steric hindrance.

On the basis of comparison of the magnitudes of the interaction energies of the two most stable states of the binary system, the oxidation reaction of indane is predicted to take place preferentially at its $C_{(1)}$ atom. This prediction coincides very well with the experimental results.

The resonance energy changes were found to be 7.83 and 2.92 eV when O_2 was brought nearer to the $C_{(1)}$ and $C_{(2)}$ atom, respectively. This result provides further support for the hydroxylation of indane at its $C_{(1)}$ atom in preference to its $C_{(2)}$ atom.

Calculations

All calculations were carried out on a FACOM M-190 computer at the Computation Center of Kyushu University.

The following values for bond lengths (Å), bond angles (°) and dihedral angles (°) of 1,2,3,4-tetrahydronaphthalene and indane were based on the reported electron diffraction data for cyclohexene[14] and cyclopentene[15] and on the X-ray crystallographic analysis of estriol.[16] In this section, the carbon atom bearing the number *i*, $C_{(i)}$, is expressed simply by the symbol *i*. The expressions *i-j*, *i-j-k* and (*i*, *j*, *k*)-(*l*, *m*, *n*) denote the bond between two atoms *i* and *j*, the bond angle defined by three atoms *i*, *j* and *k*, and the dihedral angle between the planes defined by three atoms *i*, *j* and *k* and by three atoms *l*, *m* and *n*, respectively.

1,2,3,4-Tetrahydronaphthalene

Bond lengths. 1-2, 1.517; 2-3, 1.533; 8a-1, 1.504; C-C of aromatic ring, 1.395. Bond angles. 4a-8a-1, 121.9; 8a-1-2, 113.6; 1-2-3, 111.1; H-1-H, 105.6; 8a-1-H, 109.33; H-2-H, 109.2; 1-2-H, 109.13. Dihedral angles. (4a, 8a, 1)-(8a, 1, 2), 15.2; (8a, 1, 2)-(1, 2, 3), 44.57.

Indane

Bond lengths. 1-2, 1.546; 7a-1, 1.519; C–C of aromatic ring, 1.395. Bond angles. 3a-7a-1, 111.0; 7a-1-2, 101.36; 1-2-3, 107.2; 7a-1-H, 111.46; 1-2-H, 110.04. Dihedral angle. (3a, 7a, 1)-(7a, 1, 2), 12.87.

Other bond lengths and bond angles were taken from the literature.[12] Bond lengths and bond angles of the porphine ring, used in the geometrical examination, were taken from the literature,[17] and the Fe–$O_{(2)}$ bond was assumed to be perpendicular to the heme plane.

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