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Spectroscopic Investigation on the Mechanisms of Oxygenation Reactions of Skatole Catalyzed by Co(II)(p-OCH$_3$)TPP and ClCo(III)(p-OCH$_3$)TPP

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Visible absorption spectra of 5,10,15,20-tetra(p-methoxyphenyl)porphyrinatocobalt(II) [Co(II)(p-OCH$_3$)TPP] and 5,10,15,20-tetra(p-methoxyphenyl)porphyrinatocobalt(III)chloride [ClCo(III)(p-OCH$_3$)TPP] were measured in toluene at room temperature in the absence or presence of O$_2$. Changes of the spectra due to the presence of skatole and/or pyridine were investigated.

The spectra of four-coordinate cobalt complexes of 5,10,15,20-tetra(p-methoxyphenyl)-porphyrin were found to be practically indistinguishable from those of five-coordinate complexes, but clearly different from those of six-coordinate complexes, irrespective of the oxidation state of the central cobalt ion of complexes.

The reaction medium for the oxygenation of skatole catalyzed by Co(II)(p-OCH$_3$)TPP showed only the spectrum of a six-coordinate complex, (skatole) Co(II)(p-OCH$_3$)TPP(O$_2$). This result showed clearly that the oxygenation of skatole must proceed through a ternary complex of the type (skatole)-[Co(II)complex]-(O$_2$). The addition of pyridine to the same reaction medium was found to produce a six-coordinate complex, (skatole)Co(II)(p-OCH$_3$)TPP(pyridine), irreversibly. This explains why the oxygenation of skatole is remarkably repressed upon addition of a Lewis base such as pyridine to the reaction medium.

The spectrum of the reaction medium for the oxygenation of skatole catalyzed by ClCo(III) (p-OCH$_3$)TPP was found to consist of the superposed spectra of two six-coordinate complexes, ClCo(III)(p-OCH$_3$)TPP(O$_2$) and ClCo(III)(p-OCH$_3$)TPP(skatole). Therefore, the oxygenation of skatole apparently proceeds through the interaction between these two six-coordinate complexes.

Keywords——visible absorption spectrum; 5,10,15,20-tetra(p-methoxyphenyl)porphyrinatocobalt(II); 5,10,15,20-tetra(p-methoxyphenyl)porphyrinatocobalt(III)chloride; skatole; pyridine; tryptophan 2,3-dioxygenase; Soret band; Q band; oxygenation; reaction mechanism

N,N'-ethylenebis(salicylideniminato)cobalt(II) [Co(II)(salen)],[1)] N,N'-ethylenebis-(acetylacetoniminato)cobalt(II) [Co(II)(acacen)],[2)] 5,10,15,20-tetraphenylporphyrinatocobalt(II) [Co(II)TPP],[3)] 5,10,15,20-tetraphenylporphyrinatocobalt(III)bromide [BrCo(III)-TPP],[4)] 5,10,15,20-tetra(p-methoxyphenyl)porphyrinatocobalt(II) [Co(II) (p-OCH$_3$)TPP],[5)] phthalocyaninatocobalt(II) [Co(II)Pc],[5)] and several other metal complexes are known to be usable as models of catalysis by tryptophan 2,3-dioxygenase. The mechanism of the model oxygenations, however, is in dispute.[6)]

We have proposed that the model reaction proceeds through a ternary complex of the type skatole-[Co(II)complex]-O$_2$, because the oxygenations of skatole catalyzed by Co(II)(salen), Co(II)(acacen), Co(II)(p-OCH$_3$)TPP and Co(II)Pc were all observed to be remarkably repressed upon addition of a Lewis base such as pyridine, imidazole or N-methylimidazole to the reaction medium.[5)]

In a continuation of our investigation, we found that the existence of the ternary complex in the reaction medium for the oxygenation of skatole catalyzed by Co(II)(*p*-OCH_3)TPP can be detected spectroscopically. This paper describes the results. 5,10,15,20-Tetra(*p*-methoxyphenyl)porphyrinatocobalt(III)chloride [ClCo(III)(*p*-OCH_3)TPP] was found to catalyze the oxygenation of skatole in this work, and a mechanism for this reaction is proposed.

Results and Discussion

Various experimental data[7-10] suggest that the formation of a five-coordinate complex from a four-coordinate metal complex of porphyrin will not be accompanied by any remarkable shift of the Soret band or the Q band, but the formation of a six-coordinate complex will be accompanied not only by significant shifts of the Soret band and the Q band but also by splitting of the latter band, irrespective of the oxidation state of the central metal ion of the complex. Therefore, it was expected that the presence of a ternary, six-coordinate complex in the reaction medium for the oxygenation of skatole catalyzed by Co(II)(*p*-OCH_3)TPP could be detected spectroscopically. The visible absorption spectrum of Co(II)(*p*-OCH_3)TPP was measured in toluene, which was considered not to coordinate to the catalyst. The spectrum of ClCo(III)(*p*-OCH_3)TPP was also measured.

Visible Absorption Spectra of Co(II)(*p*-OCH_3)TPP and ClCo(III)(*p*-OCH_3)TPP in Toluene

An intense and sharp Soret band and a fairly sharp Q band were observed at 413 and 527 nm, respectively, in the spectrum of a degassed toluene solution of Co(II)(*p*-OCH_3)TPP (5×10^{-6} M)[11] (Fig. 1).

The presence of pyridine (1×10^{-2} M)[12] or skatole (1×10^{-2} M)[12] in the degassed toluene solution of Co(II)(*p*-OCH_3)TPP (5×10^{-6} M) was found to have no significant influence on the spectrum of the latter compound. The result obtained in the presence of pyridine indicates that the spectrum of the five-coordinate complex is indistinguishable from that of the four-coordinate complex, Co(II)(*p*-OCH_3)TPP, because pyridine is known to coordinate to the catalyst to form a five-coordinate complex, Co(II)(*p*-OCH_3)TPP(pyridine), but not a six-coordinate complex at room temperature.[13] With regard to the coordination of skatole to the catalyst, no experimental evidence has yet been presented, but the result mentioned above shows that skatole does not coordinate to the catalyst to form a six-coordinate complex at

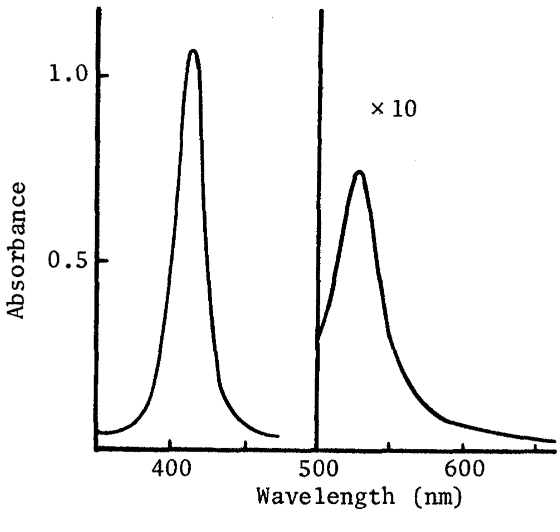

Fig. 1. Absorption Spectrum of a Degassed Toluene Solution of Co(II)(*p*-OCH_3)TPP (5×10^{-6} M)

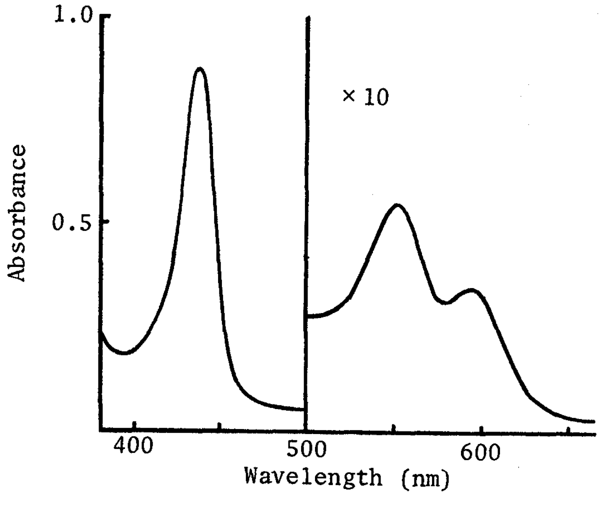

Fig. 2. Absorption Spectrum of a Degassed Toluene Solution of Co(II)(*p*-OCH_3)TPP (5×10^{-6} M) in the Presence of Pyridine (1×10^{-2} M) and Skatole (1×10^{-2} M)

room temperature.

The degassed toluene solution of Co(II)(*p*-OCH_3)TPP did not show any spectral change on standing at room temperature in the absence or in the presence of pyridine or skatole. However, the solution showed a gradual spectral change in the presence of skatole when the solution was exposed to air. Namely, the peak of the Soret band at 413 nm and the peak of the Q band at 527 nm became gradually weaker, a new peak appeared in the Soret band region at 438 nm and the Q band gradually split into two peaks at 555 and 596 nm. The peak positions of these new bands coincided very well with those of the Soret band and the Q band of the six-coordinate complex, as mentioned below.

A degassed toluene solution of Co(II)(*p*-OCH_3)TPP (5×10^{-6} M) showed an intense and sharp Soret band at 438 nm and a Q band having two peaks at 555 and 596 nm in the presence of both pyridine (1×10^{-2} M) and skatole (1×10^{-2} M) (Fig. 2). This spectrum is presumably the spectrum of the six-coordinate complex, (pyridine)Co(II)(*p*-OCH_3)TPP(skatole).

The spectrum of a degassed toluene solution of ClCo(III)(*p*-OCH_3)TPP (5×10^{-6} M) was found to be almost indistinguishable from that of Co(II)(*p*-OCH_3)TPP measured in degassed toluene. This result indicates that the spectrum of five-coordinate cobaltic complex is practically identical with that of four-coordinate cobaltous complex of 5,10,15,20-tetra(*p*-methoxyphenyl)porphyrin.

The spectrum of a degassed toluene solution of ClCo(III)(*p*-OCH_3)TPP (5×10^{-6} M) measured in the presence of pyridine (1×10^{-2} M) was found to be almost indistinguishable from that shown in Fig. 2, and the spectrum did not change at all on standing at room temperature. This result indicates that pyridine coordinates to the catalyst to form a six-coordinate complex, ClCo(III)(*p*-OCH_3)TPP(pyridine).

Degassed toluene solutions of ClCo(III)(*p*-OCH_3)TPP and skatole were mixed so as to make the concentrations of the two compounds in the mixture 5×10^{-6} and 1×10^{-2} M, respectively, and the mixture was kept standing in the degassed state at room temperature. The spectrum measured immediately after the preparation of the mixture is shown by curve 1 of Fig. 3. The band at 438 nm was fairly broad and was accompanied by a shoulder at about

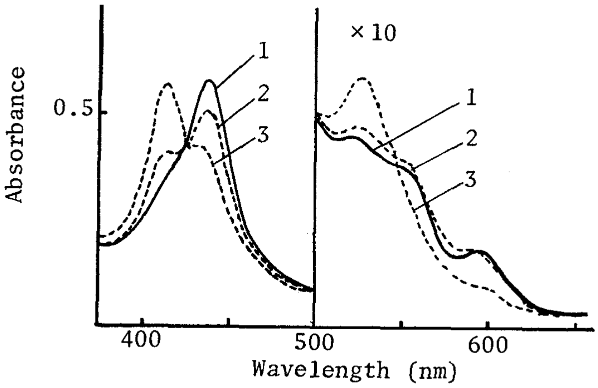

Fig. 3. Spectral Change of a Mixture of a Degassed Toluene Solutions of ClCo(III)(*p*-OCH_3)TPP (5×10^{-6} M) and of Skatole (1×10^{-2} M)

Curve 1: measured just after mixing.
Curves 2 and 3: measured 3 and 12 h after mixing, respectively.

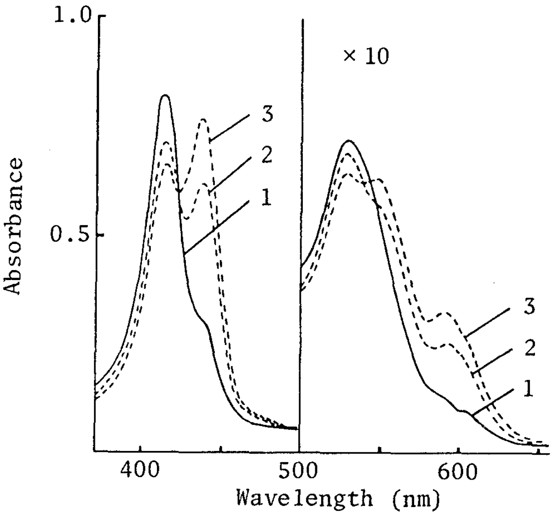

Fig. 4. Spectral Change of an N_2-Treated Toluene Solution of ClCo(III)(*p*-OCH_3)TPP (5×10^{-6} M)

Curve 1: measured before O_2 bubbling.
Curves 2 and 3: measured 20 and 30 min after the beginning of O_2 bubbling, respectively.

413 nm, and the Q band consisted of two peaks at 527 and 596 nm and one shoulder at about 555 nm. This result indicates that both the six-coordinate complex, ClCo(III)(*p*-OCH_3)TPP(skatole), and the five-coordinate complex, ClCo(III)(*p*-OCH_3)TPP, exist in the mixture, and that the amount of the former complex is much greater than that of the latter.

Curves 2 and 3 of Fig. 3 show the spectra measured 3 and 12 h after mixing, respectively. The spectral change shown by these curves may be due to the slow progression of the reaction expressed by eq. 1.

$$\mathrm{ClCo(III)}(p\text{-}\mathrm{OCH_3})\mathrm{TPP(skatole)} \longrightarrow [\mathrm{Co(III)}(p\text{-}\mathrm{OCH_3})\mathrm{TPP}]^{+}(\text{skatole anion}) + \mathrm{HCl} \qquad (1)$$

Oxygen gas was bubbled continuously at room temperature through an N_2-treated toluene solution of ClCo(III)(*p*-OCH_3)TPP (5×10^{-6} M), and the spectrum of this solution was measured periodically. Here, N_2-treated toluene means toluene that had been made practically free from air by bubbling N_2 gas through it. Curve 1 of Fig. 4 shows the spectrum of the solution measured before the beginning of O_2 bubbling. This spectrum indicates that most of the catalyst was left intact in the solution but some was converted to the six-coordinate complex, ClCo(III)(*p*-OCH_3)TPP(O_2). The formation of the latter complex is considered to be due to the coordination of a small amount of O_2 remaining in the solvent. As O_2 bubbling went on, the spectrum of the solution changed rather quickly to that shown by curve 3 of Fig. 4. Thereafter, the solution did not show any further spectral change. The spectrum measured 30 min after the beginning of O_2 bubbling (curve 3, Fig. 4) had two and three absorption bands in the Soret band region and the Q band region, respectively, and the peak at 438 nm was higher than that at 413 nm. This result indicates that roughly half of the catalyst remains intact even if a large amount of O_2 is supplied to the solution.

Spectroscopic Investigation on the Mechanism of Oxygenation of Skatole Catalyzed by Co(II)(*p*-OCH_3)TPP

An O_2-treated toluene solution of Co(II)(*p*-OCH_3)TPP (1×10^{-5} M) was mixed with an equal volume of O_2-treated toluene solution of skatole (2×10^{-2} M). Here, O_2-treated toluene means the toluene that had been almost saturated with O_2 by bubbling O_2 gas through the solvent. A sample of the reaction mixture was separated from the mixture and kept standing in a stoppered flask at room temperature. This sample is designated as reaction medium A. The remainder of the reaction mixture was kept in a flask, and O_2 gas was bubbled through the mixture continuously at room temperature. This reaction mixture is designated as reaction medium B.

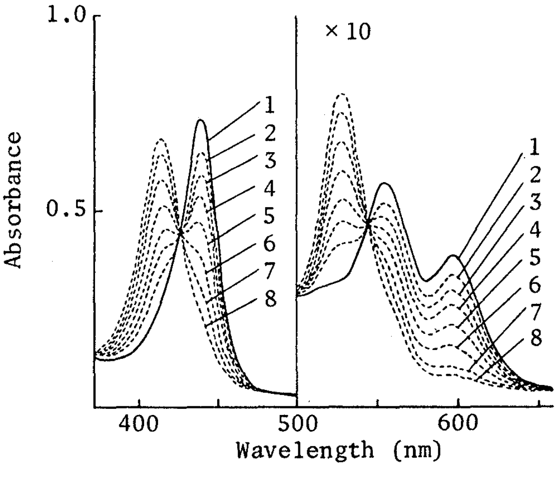

Fig. 5. Spectral Change of Reaction Medium A

Curve 1: measured just after the preparation of the medium.

Curves 2—8: measured 9, 17, 21, 25, 34, 53, and 79 min after the preparation of the medium, respectively.

The spectrum of reaction medium A measured just after the mixing of the two solutions (curve 1, Fig. 5) was almost indistinguishable from that shown in Fig. 2. This result indicates that both skatole and O_2 coordinate very quickly to the catalyst to form the six-coordinate complex, (skatole)Co(II)(*p*-OCH_3)TPP(O_2). As the standing time increased, reaction medium A showed a fairly rapid spectral change (Fig. 5). The shape of the spectrum measured 79 min after the mixing of the two solutions (curve 8, Fig. 5) resembled that of the spectrum shown in Fig. 1, although both the Soret band and the Q band of the former spectrum were somewhat broader than those of the latter spectrum.

The spectrum of reaction medium B was almost identical with the initial spectrum of reaction medium A even 5 h after the beginning of O_2 bubbling. When the O_2 bubbling was stopped, reaction medium B showed a spectral change quite similar to that of reaction medium A, as the time of standing in a stoppered flask increased.

When reaction medium A showed the spectrum corresponding to curve 6 of Fig. 5, the medium was cooled[14)] in an ice-bath and O_2 gas was bubbled through it for 30 min. As the time of O_2 bubbling increased, the spectrum of the medium was observed to change in the reverse direction to that shown in Fig. 5, and finally it became almost indistinguishable from that shown by curve 2 of Fig. 5. After the end of the O_2 bubbling, the spectrum of this medium was observed to change again in the same direction as that shown in Fig. 5. The same phenomenon was also observed upon O_2 bubbling through reaction medium A when it showed a spectrum corresponding to curve 8 of Fig. 5.

The data presented above are consistent with the following representation of the oxygenation of skatole catalyzed by Co(II)(*p*-OCH_3)TPP (Chart 1).

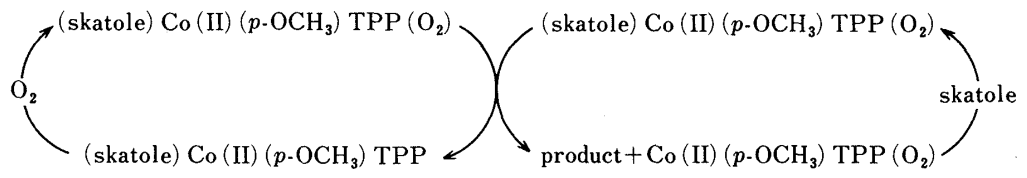

Chart 1

A concentrated O_2-treated toluene solution of pyridine was added to reaction medium A when the medium had the spectrum shown by curve 8 of Fig. 5 so as to make the concentration of pyridine in the resulting mixture approximately 1×10^{-2} M. The spectrum of reaction medium A changed quickly, becoming similar to curve 1 of Fig. 5, and afterwards it did not change further. Pyridine was also added to reaction medium A by the same procedure as employed in the above case when the medium had a spectrum corresponding to curve 1 of Fig. 5. The spectrum of the reaction medium A did not change at all with the addition of pyridine or on standing. These results are considered to be due to the formation of (skatole)Co(II)(*p*-OCH_3)TPP(pyridine), and explain why the oxygenation of skatole catalyzed by Co(II)(*p*-OCH_3)TPP was repressed upon addition of a Lewis base such as pyridine to the reaction medium.

Spectroscopic Investigation on the Mechanism of Oxygenation of Skatole Catalyzed by ClCo(III)(*p*-OCH_3)TPP

O_2-Treated toluene solutions of ClCo(III)(*p*-OCH_3)TPP and of skatole were mixed so as to make the concentrations of the two compounds in the mixture 5×10^{-6} and 1×10^{-2} M, respectively. A sample was taken from the reaction mixture, then O_2 gas was bubbled through the remainder of the reaction mixture continuously at room temperature. The sample was kept standing in a stoppered flask at room temperature. The sample and the remainder of the reaction mixture are designated as reaction medium C and reaction medium D, respectively.

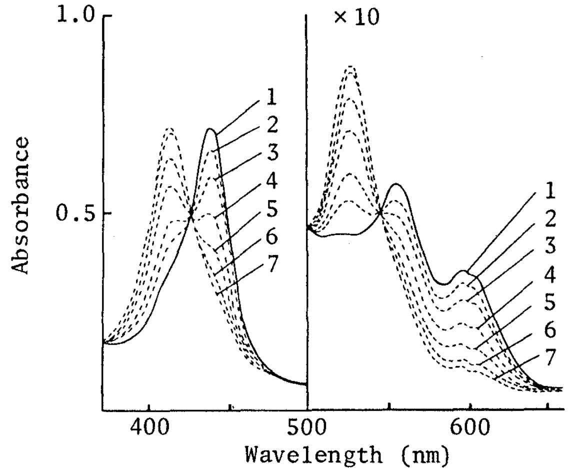

Fig. 6. Spectral Change of Reaction Medium C

Curve 1: measured just after the preparation of the medium.

Curves 2—7: measured 9, 13, 20, 29, 50, and 77 min after the preparation of the medium, respectively.

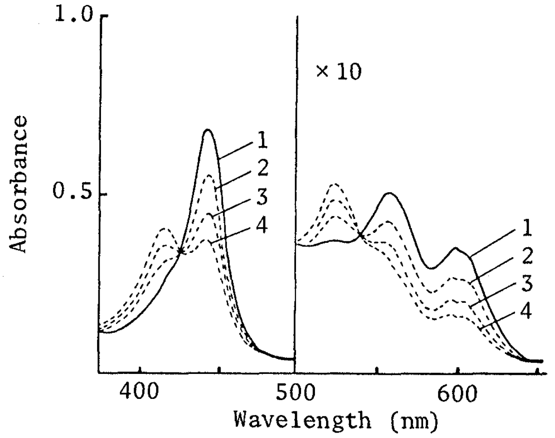

Fig. 7. Spectral Change of a Toluene Solution of ClCo(III)(*p*-OCH$_3$)TPP (5×10^{-6} M) and Skatole (1×10^{-2} M)

Curve 1: measured just after mixing.

Curves 2—4: measured 30, 60, and 120 min after the beginning of O$_2$ bubbling, respectively.

Reaction medium C was found to show a spectral change analogous to that of reaction medium A, as the time of standing increased (Fig. 6). Its initial spectrum (curve 1, Fig. 6) closely resembled that shown in Fig. 2, except for the two weak shoulders at 413 and about 527 nm. This result indicates that a large amount of six-coordinate complex and a small amount of intact catalyst existed initially in reaction medium C. Even if O$_2$ was bubbled through a toluene solution of ClCo(III)(*p*-OCH$_3$)TPP for more than 30 min, roughly half the catalyst remained intact in the solution at room temperature. It was also found that, even if a 2000-fold excess of skatole over ClCo(III)(*p*-OCH$_3$)TPP were present in a toluene solution of the catalyst, a small amount of catalyst remained intact in the solution at room temperature. Therefore, the six-coordinate complexes observed spectroscopically in reaction medium C must be ClCo(III)(*p*-OCH$_3$)TPP(O$_2$) and ClCo(III)(*p*-OCH$_3$)TPP(skatole).

In contrast to the case of reaction medium B, the spectrum of reaction medium D was observed to change gradually (Fig. 7).[15] This spectral change is considered to be due to the slow formation of a five-coordinate complex, presumably having the structure [Co(III)(*p*-OCH$_3$)TPP]$^+$(skatole anion).

As in the case of reaction medium A, reaction medium C was cooled in an ice-bath and O$_2$ was bubbled through it for 30 min when the medium had a spectrum corresponding to curve 5 of Fig. 6. The spectrum of the medium began to change in the reverse direction to that shown in Fig. 6. After the end of the O$_2$ bubbling, the spectrum of the medium was found to change in the same direction as that shown in Fig. 6.

The experimental results mentioned above are consistent with the following representation of the oxygenation of skatole catalyzed by ClCo(III)(*p*-OCH$_3$)TPP (Chart 2).

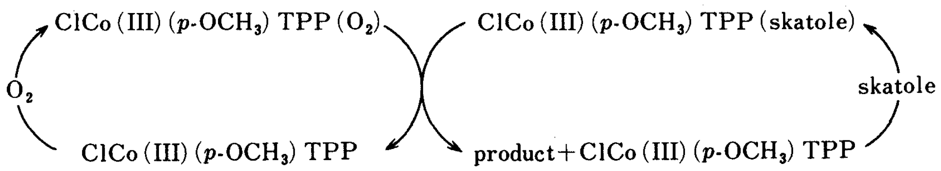

Chart 2

A concentrated O_2-treated toluene solution of pyridine was added to reaction medium D when the medium had a spectrum corresponding to curve 6 of Fig. 7 so as to make the concentration of pyridine in the resulting mixture approximately 1×10^{-2} M. The spectrum of the medium changed quickly, becoming very similar to that shown in Fig. 2, and afterwards it did not change at all. This spectral change is considered to be due to the formation of two six-coordinate complexes, ClCo(III)(*p*-OCH_3)TPP(pyridine) and (pyridine)[Co(III)(*p*-OCH_3)-TPP]$^+$(skatole anion).

Experimental

Chemicals——Skatole (reagent grade), pyridine (reagent grade), and toluene (for spectroscopic use) were used without further purification.

5,10,15,20-Tetra(*p*-methoxyphenyl)porphyrinatocobalt(III)chloride [ClCo(III)(*p*-OCH_3)TPP]——Air was bubbled with vigorous stirring through a suspension of 5,10,15,20-tetra(*p*-methoxyphenyl)porphyrinatocobalt(II) [Co(II)(*p*-OCH_3)TPP] powder (1.0 g) in a mixture of MeOH (2.0 l) and conc. HCl (10 ml) overnight. After the removal of a small amount of insoluble substances by filtration, the filtrate was concentrated *in vacuo* to about 100 ml at a temperature lower than 50 °C. Addition of water (200 ml) gave a crystalline product. The product was washed with water and dried *in vacuo* at room temperature. The yield was 0.74 g. Recrystallization of the product (200 mg) from benzene–*n*-hexane (1 : 2) afforded deep-bluish-violet prisms (80 mg). *Anal.* Calcd for $C_{48}H_{36}ClCoN_4O_4$: C, 69.69; H, 4.39; N, 6.77. Found: C, 69.34; H, 4.51; N, 6.69. UV $\lambda_{\max}^{\text{toluene}}$ nm (log ε): 413 (4.92), 527 (3.83). Magnetic susceptibility was measured by the Faraday method at 21 °C; the complex is diamagnetic.

Catalytic Oxygenation of Skatole by ClCo(III)(*p*-OCH_3)TPP——Skatole (52.5 mg, 2×10^{-2} M) was dissolved in 20 ml of a CH_2Cl_2 solution of ClCo(III)(*p*-OCH_3)TPP (6.6 mg, 4×10^{-4} M), and O_2 gas was bubbled through the reaction mixture for 7 h at room temperature. The resulting mixture was concentrated *in vacuo* to about 10 ml, and separated on a silica-gel column (3.7 g) using CH_2Cl_2 as the elution solvent. Skatole was recovered from the first colorless eluate. The second faint-greenish-yellow eluate was found to contain a very small amount of *o*-aminoacetophenone by thin layer chromatography (TLC). The third dark-yellow eluate was found to contain the catalyst by TLC. The fourth faint-yellow eluate was evaporated *in vacuo*, and the residue was dissolved in 25 ml of petroleum ether. The yellowish solution was decolorized with activated charcoal, and concentrated to about 4 ml to afford colorless needles of mp 79—79.5 °C. The infrared (IR) spectrum of the crystals was identical with that of an authentic sample of *o*-formamidoacetophenone. These results indicate that skatole was oxygenated catalytically by ClCo(III)(*p*-OCH_3)TPP.

Preparation of Degassed Toluene Solutions——Toluene was boiled under reflux for a few h and allowed to cool to room temperature with continuous bubbling of N_2 gas through the solvent to prepare N_2-treated toluene. Co(II)(*p*-OCH_3)TPP, ClCo(III)(*p*-OCH_3)TPP, skatole and pyridine were dissolved in N_2-treated toluene separately in a dry-box filled with N_2 gas. Each of these solutions was degassed by the freeze-thaw pumping cycle in a vacuum line ($< 1 \times 10^{-6}$ Torr). The mixing of two or three solutions and the transfer of a solution or a mixture to an optical cell were all performed in the absence of air.

Spectral Measurements——All spectra were recorded on a Hitachi 200-20 spectrometer at room temperature.

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11) Dilute toluene solutions of Co(II)(*p*-OCH_3)TPP and ClCo(III)(*p*-OCH_3)TPP were used throughout the work in order to make the spectra of these solutions directly measurable.

12) The molar ratio of pyridine or skatole to Co(II)(*p*-OCH_3)TPP or ClCo(III)(*p*-OCH_3)TPP was 2000 to 1 throughout the work in order to hasten the coordination reaction; otherwise, the reaction proceeds too slowly for convenient observation of the whole spectral change due to the reaction.

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14) Reaction medium A was cooled in order to reduce the reaction velocity and also to increase the O_2 solubility in the medium.

15) The O_2 bubbling through reaction medium D was accompanied by evaporation of the solvent of the medium. Therefore, O_2-treated toluene was added to the medium at each sampling time in order to compensate for the solvent lost.