CHEMICAL & PHARMACEUTICAL BULLETIN

Vol. 29, No. 6 June 1981

Regular Articles

[Chem. Pharm. Bull. 29(6)1495—1500(1981)]

Studies on the Catalytic Oxidation Reactions of Skatole using Cobalt Complexes

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(Received September 1, 1980)

Oxygenation reactions of skatole catalyzed by N,N′-ethylenebis(salicylideniminato)-Co(II) [Co(salen)], N,N′-ethylenebis(acetylacetoniminato)Co(II) [Co(acacen)], Co(II)-complex of α,β,γ,δ-tetra(*p*-methoxyphenyl)porphine [Co(*p*-OCH_3)TPP] and Co(II)-complex of phthalocyanine (CoPc) were investigated.

Remarkable repression of the oxygenation was observed upon addition of a Lewis base such as pyridine, imidazole or N-methylimidazole to the reaction medium. This phenomenon was thought to indicate that the oxygenation proceeds through a ternary complex of the type skatole-Co-complex-O_2.

The reaction rate constants and activation energies of all the reactions were also evaluated.

Keywords——skatole; N,N′-ethylenebis(salicylideniminato)Co(II); N,N′-ethylenebis-(acetylacetoniminato)Co(II); Co(II)-complex of α,β,γ,δ-tetra(*p*-methoxyphenyl)porphine; Co(II)-complex of phthalocyanine; pyridine; imidazole; N-methylimidazole; ternary complex

In recent years, much work has been done on the structures and functions of various enzymes, and model enzymatic reactions using metal complexes have also attracted much interest. Information obtained from model enzymatic reactions may not necessarily be directly applicable to enzymatic reactions, but should still be helpful to the understanding of enzymatic reactions, because the structures and functions of protein moieties of many important enzymes are not generally well-known.

In the case of biological oxidations, molecular oxygen participates in the reaction in its activated state, produced by the catalytic action of an oxygenase, which may be a mono-oxygenase or a dioxygenase.

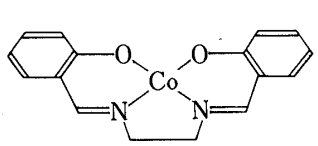
Tryptophan 2,3-dioxygenase, to which *l*-tryptophan pyrrolase and indoleamine dioxygenase belong, is a typical dioxygenase containing protoheme IX. This enzyme catalyzes the oxidative cleavage of the heterocyclic ring of 3-substituted indoles at their $C_{(2)}$–$C_{(3)}$ bonds. A representative example of this reaction is the transformation of *l*-tryptophan to formylkynurenine, the first step in one of the metabolic pathways of the former compound.

(1)

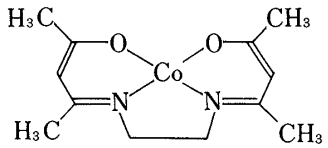
Chart 1

In 1975, Nishinaga[1] presented a model of tryptophan 2,3-dioxygenase. He showed that N,N′-ethylenebis(salicylideniminato)Co(II) [Co(salen)] catalyzes the oxidative cleavage reaction of 3-substituted indoles related to tryptophan to give the corresponding *o*-formylaminoacetophenone derivatives in good yield. Afterwords, Co(II)-, Cu(II)- and Ni(II)- complexes of α,β,γ,δ-tetraphenylporphine (CoTPP, CuTPP and NiTPP) and Co(II)-complex of deuteroporphyrin dimethylester (CoDPDME),[2] Mn(II)-complex of phthalocyanine (MnPc)[3] and N,N-ethylenebis (acetylacetoniminato)Co(II)[Co(acacen)] and N,N′-ethylenebis(benzoylacetoniminato)Co(II) [Co(bzacen)][4] were also found to catalyze the same reaction.

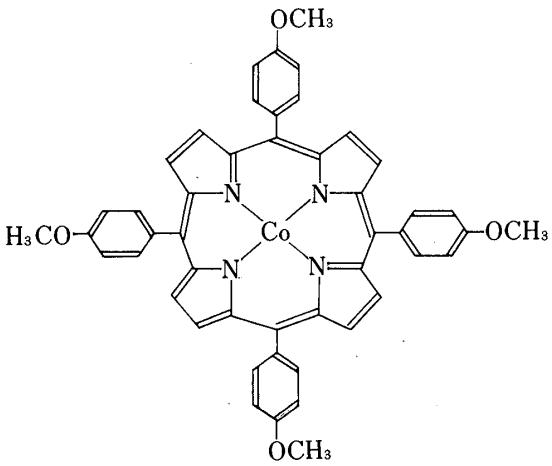
With regard to the mechanism of the model oxygenation, two suggestions have been presented. Namely, Nishinaga[1] suggested that the Co(salen)-catalyzed oxygenation requires an electron transfer process involving transient formation of a Co–O_2 complex with 3-substituted indole as an axial ligand, and Uchida *et al.*[3] proposed that skatole activated by MnPc–O_2^- reacts with the oxygen in another ternary complex, MnPc, O_2 and skatole, producing *o*-formylaminoacetophenone. In order to obtain clear evidence of the mechanism of the model oxygenation reactions, we began to investigate the metal-complex-catalyzed oxygenation of skatole in considerably detail. This paper describes the results. The catalysts we selected were Co(salen) and Co(acacen), whose catalytic activities are already known, and two new Co-complexes, Co(II)-complex of phthalocyanine (CoPc) and Co(II)-complex of α,β,γ,δ-tetra(*p*-methoxyphenyl)porphine [Co(*p*-OCH_3)TPP].



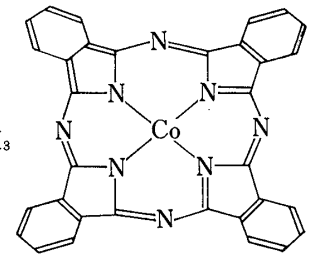
Co (salen)



Co (acacen)



Co (*p*-OCH_3)TPP



CoPc

Chart 2

Experimental

Metal Complexes——Co(salen),[5] Co(acacen),[6] Co(*p*-OCH_3)TPP[7] and CoPc[8] were prepared by the cited methods.

Chemicals——Skatole (reagent grade) and *p*-dimethylaminobenzaldehyde (for biochemical use) were used without any further purification. *o*-Formylaminoacetophenone was prepared by the method of Dolby.[9]

Modified Ehrlich's Reagent——*p*-Dimethylaminobenzaldehyde (10 g) was dissolved in a mixture of 1000 ml of EtOH and 8 ml of conc. H_2SO_4 just before use.[10]

General Procedure——Water maintained at a constant temperature by means of a temperature control apparatus (Sharp TE-4) was circulated in the water jacket of a handmade double-walled reaction vessel, and oxygen gas was blown through a solution of Co-complex in 40 ml of MeOH or CH_2Cl_2 in the vessel. Thereafter, an oxygen-saturated solution of skatole in 10 ml of the same solvent was added through an inlet valve fitted to the vessel. The reaction mixture was stirred vigorously with a magnetic stirrer under an atmosphere of oxygen gas at about 760 Torr. Small portions of the reaction mixture, 1.0 ml in each case, were withdrawn through a small outlet valve immediately and every 30 min after the start of the reaction. Each portion was passed through a column of 10 ml of ion exchange resin [Dowex 50W-8 (H^+ form)] packed with MeOH in a glass tube [1.0 cm(ϕ) × 12 cm] to remove the catalyst[11] immediately after sampling. The column was eluted with MeOH and 25.0 ml of the eluate was subjected to the colorimetric determination of skatole remaining in the reaction mixture.

Skatole develops an intense reddish-purple color (λ_{max} 582 nm with a shoulder at 547 nm) with the modified Ehrlich's reagent. Both *o*-formylaminoacetophenone and *o*-aminoacetophenone, the oxidation products of skatole catalyzed by Co-complexes,[1-4] develop a faint yellow color (λ_{max} 459 nm) with the same reagent, but it was ascertained by preliminary experiments that the coexistence of these compounds does not interfere with the quantitative determination of skatole in a solution. At the beginning of the color reaction the absorbance at 582 nm increases rather rapidly on standing at room temperature. Within one hr the absorbance reaches the maximum value, and subsequently remains unchanged. The absorbance read after one hr of standing was found to obey Lambert-Beer's law. The colorimetry of skatole was performed as follows: Each of three small portions (0.5 ml each) of the eluate was mixed well with 2.5 ml of the modified Ehrlich's reagent and left to stand at room temperature for one hr, then the absorbance of the reaction mixture at 582 nm was read on a Union SM-401 spectrometer. The average absorbance of three separate measurements was used.

Results and Discussion

Skatole was selected as the substrate for the model oxygenation because tryptophan is hardly soluble in the organic solvents necessary to dissolve the catalysts. On the other hand, skatole is soluble in organic solvents and its concentration in the reaction mixture is easily determinable without separation from its oxidation products, *o*-formylaminoacetophenone and *o*-aminoacetophenone.

Co(salen) and Co(acacen) are soluble in MeOH. Co(*p*-OCH_3)TPP and CoPc are hardly soluble in MeOH but are soluble in CH_2Cl_2 to a sufficient extent for the present purpose. Therefore, MeOH was used as the solvent in the reactions using the former catalysts and CH_2Cl_2 as that in the reactions using the latter catalysts.

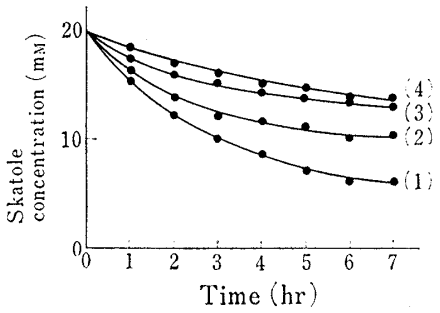


Fig. 1. Change of Skatole Concentration with Reaction Time

Reaction conditions: skatole, 20 mM; catalyst (CoPc), (1) 0.4 mM, (2) 0.2 mM, (3) 0.1 mM and (4) 0.05 mM; solvent, CH_2Cl_2; temperature, 25°.

Figure 1 shows, as an example of our results, how the amount of skatole in the reaction mixture decreased with reaction time when CoPc was employed as the catalyst. In all cases examined, the logarithm of the ratio of $[skatole]^0$ to $[skatole]^t$ was found to correlate linearly with the reaction time, t. Here, brackets indicate the concentration and superscripts 0 and t denote reaction times of zero and t min. These results mean that the model oxygenation examined in this investigation can be treated as a pseudo first order reaction with respect to skatole.

In order to determine the order of the reaction with respect to catalyst, the initial concentration of skatole was kept constant and the reaction was carried out with various catalyst concentrations at 25°. The initial velocities read from the plots, one of which is shown in Fig. 1, are listed in Table I. In each case, the logarithm of the initial velocity was found to correlate linearly with the logarithm of the catalyst concentration. The reaction orders of

Table I. Initial Velocities of Model Oxidation Reactions

Catalyst	Solvent	Initial concentration of skatole (mM)	Concentration of catalyst (mM)	Initial velocity (mM sec^{-1})
Co(salen)	MeOH	40	20	5.8×10^{-3}
			10	2.2×10^{-3}
			5	8.5×10^{-4}
			2.5	3.8×10^{-4}
Co(acacen)	MeOH	40	20	8.8×10^{-3}
			10	5.2×10^{-3}
			5	2.0×10^{-3}
			2.5	1.2×10^{-3}
Co(*p*-OCH_3)TPP	CH_2Cl_2	20	1.5	7.3×10^{-4}
			1.0	5.7×10^{-4}
			0.5	5.0×10^{-4}
			0.25	4.0×10^{-4}
			0.125	3.5×10^{-4}
			0.0625	2.5×10^{-4}
CoPc	CH_2Cl_2	20	0.4	1.3×10^{-3}
			0.2	1.0×10^{-3}
			0.1	6.7×10^{-4}
			0.05	4.5×10^{-4}

Table II. Reaction Rate Constants and Activation Energies

Catalyst and its concentration (mM)	Solvent	Initial concentration of skatole (mM)	Temperature (°C)	Reaction rate constant (sec^{-1})	Activation energy (kJ mol^{-1})
Co(salen) 10	MeOH	40	15	1.67×10^{-5}	76.0
			25	4.37×10^{-5}	
			35	1.25×10^{-4}	
Co(acacen) 10	MeOH	40	15	4.27×10^{-5}	25.3
			25	5.07×10^{-5}	
			35	8.33×10^{-5}	
Co(*p*-OCH_3)TPP 1.5	CH_2Cl_2	20	5	2.38×10^{-5}	8.3
			15	2.47×10^{-5}	
			25	3.10×10^{-5}	
CoPc 0.2	CH_2Cl_2	20	5	2.88×10^{-5}	15.2
			15	3.88×10^{-5}	
			25	4.45×10^{-5}	

the model oxygenations were calculated to be 1.3, 1.0, 0.3 and 0.5 with respect to Co(salen), Co(acacen), Co(*p*-OCH_3)TPP and CoPc, respectively. Since more detailed investigation was not carried out, these orders must be considered to be apparent ones.

If we assume that the larger the initial velocity at a given molar ratio of catalyst to skatole is, the higher the catalytic efficiency is, we can compare the efficiencies of the four kinds of catalysts with each other. However, due to the difference of solvents employed, it was only possible to compare the efficiency of Co(salen) with that of Co(acacen) and to compare the efficiency of Co(*p*-OCH_3)TPP with that of CoPc. The results shown in Table I indicate that Co(acacen) is more effective than Co(salen) and that CoPc is much more effective than Co(*p*-OCH_3)TPP in the range of molar ratio examined.

Next, the reaction rate constants and activation energies of the model oxygenations were evaluated. The results are shown in Table II.

In the oxygenation of skatole, molecular oxygen is thought to bind to the metal atom of the complex.[1-7] Therefore, the substrate molecule must bind either to the metal atom of the complex at its fifth coordination site competitively with the solvent to form a type A ternary complex, skatole-Co-complex-O_2, or directly to the oxygen molecule bound to the metal atom of the complex to form a type B ternary complex, Co-complex-O_2-skatole. It is not yet clear which type of ternary complex is involved. However, Lewis base is known to affect the reversible O_2 binding to a Co-complex and to stabilize the six-coordinate, 1:1 type oxygen adduct, base-Co-complex-O_2.[12] This phenomenon may be used to identify the actual mechanism. When a Lewis base, such as pyridine, imidazole or N-methylimidazole, is added to the model oxygenation medium, the base is thought to bind to the metal atom of the Co-complex preferentially at its fifth coordination site. If the oxygenation proceeds through the type A ternary complex, the oxygenation should be repressed by addition of Lewis base to the reaction medium because of the decrease of the amount of ternary complex. On the other hand, if the oxygenation proceeds through the type B ternary complex, the oxygenation should be accelerated because of the activation of O_2 of the ternary complex upon addition of Lewis base to the reaction medium.

In order to ascertain whether the oxygenation of skatole is repressed or accelerated by an addition of Lewis base, equimolar amounts of pyridine, imidazole and N-methylimidazole with respect to the catalysts were added to the model oxygenation media, and the reactions were carried out at 25°. At intervals, the concentration of skatole remaining in the reaction mixture was determined colorimetrically. As shown in Figs. 2—4, all the reactions examined were clearly repressed in the presence of Lewis bases. In the case of the catalyst Co(*p*-OCH_3)-TPP, the reaction was found not to proceed at all. In order to estimate the effects of Lewis bases on the model oxygenation, the decomposition ratios of skatole (defined as the ratio of the concentration of skatole initially present to that present in the reaction mixture after 4 hr of reaction) were calculated. The results are shown in Table III. These results seem to support the view that the oxygenation of skatole proceeds through the type A ternary complex. The experimental results reported by Nishinaga[1] and Uchida *et al.*[3] can also be

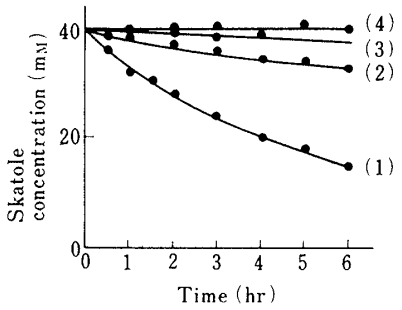


Fig. 2. Change of Skatole Concentration with Reaction Time

Reaction conditions: skatole, 40 mM; catalyst [Co (salen)], 10 mM; base, (1) none, (2) pyridine (10 mM), (3) imidazole (10 mM) and (4) N-methylimidazole (10 mM); solvent, MeOH; temperature, 25°.

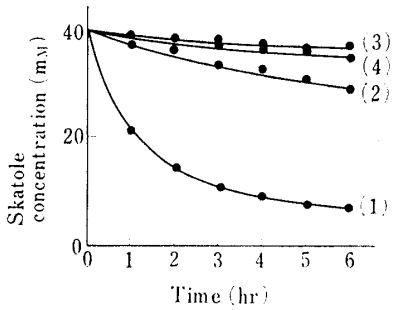


Fig. 3. Change of Skatole Concentration with Reaction Time

Reaction conditions: skatole, 40 mM; catalyst [Co (acacen)], 10 mM; base, (1) none, (2) pyridine (10 mM), (3) imidazole (10 mM) and (4) N-methylimidazole (10 mM); solvent, MeOH; temperature, 25°.

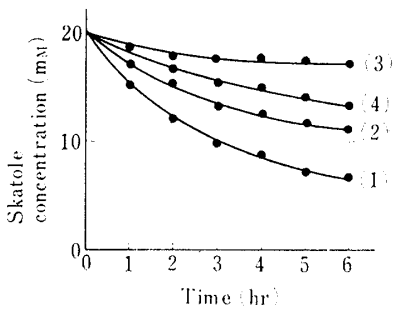


Fig. 4. Change of Skatole Concentration with Reaction Time

Reaction conditions: skatole, 20 mM; catalyst (CoPc), 0.4 mM; base, (1) none, (2) pyridine (0.4 mM), (3) imidazole (0.4 mM) and (4) N-methylimidazole (0.4 mM); solvent, CH_2Cl_2; temperature, 25°.

Table III. Decomposition Ratios of Skatole measured 4 hr after the Beginning of the Reactions

Catalyst and its concentration (mM)	Solvent	Initial concentration of skatole (mM)	Base and its concentration (mM)	Decomposition rate (%)
Co(salen) 10	MeOH	40	None	49.7
			Pyridine, 10	13.3
			Imidazole, 10	2.8
			N-Methylimidazole, 10	0
Co(acacen) 10	MeOH	40	None	77.1
			Pyridine, 10	18.2
			Imidazole, 10	6.3
			N-Methylimidazole, 10	8.0
Co(*p*-OCH_3)TPP 1.5	CH_2Cl_2	20	None	38.5
			Pyridine, 1.5	0
			Imidazole, 1.5	0
			N-Methylimidazole, 1.5	0
CoPc 0.4	CH_2Cl_2	20	None	56.3
			Pyridine, 0.4	36.2
			Imidazole, 0.4	11.9
			N-Methylimidazole, 0.4	25.7

interpreted satisfactorily in terms of the involvement of the type A ternary complex in the oxygenation.

References and Notes

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