Synthesis, Physicochemical Properties, and Amine-Oxidation Reaction of Indolequinone Derivatives as Model Compounds of Novel Organic Cofactor TTQ of Amine Dehydrogenases

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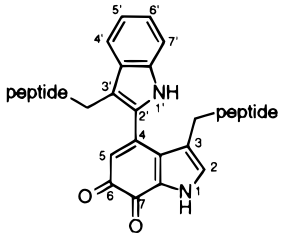
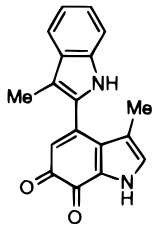
3,4-Disubstituted 6,7-indolequinones [1,3-dimethyl-4-(3′-methylindol-2′-yl)indole-6,7-dione (**2**), 3-methyl-4-phenylindole-6,7-dione (**3**), and 3,4-dimethyl-6,7-dione (**4**)] and a 3,7-disubstituted 4,5-indolequinone [3,7-dimethylindole-4,5-dione (**5**)] have been synthesized as models for the novel organic cofactor TTQ of bacterial amine dehydrogenases. The substituent and structural effects on the physicochemical properties of the quinones have been investigated in detail by comparing the spectroscopic data (UV−vis, IR, ^{1}H- and ^{13}C-NMR), pK_a values of the pyrrole proton, and the two-electron redox potentials with those of model compound **1** [3-methyl-4-(3′-methylindol-2′-yl)indole-6,7-dione] previously reported (ref 5). Reactivity of each quinone in the transamination process [iminoquinone formation (k_1), rearrangement to product−imine (k_2), and aminophenol formation (k_3)] has been investigated kinetically, revealing that the substituent and structural effects on the amine-oxidation reaction are not so significant. In the aerobic catalytic oxidation of benzylamine, however, the aromatic substituents on the quinone ring play an important role to protect the quinone from the deactivation process of a Michael-type addition by the amine, making it act as an efficient turnover catalyst.

Introduction

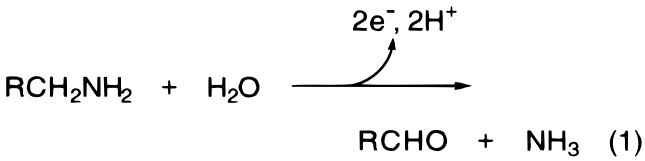
Bacterial methylamine dehydrogenase (MADH) and aromatic amine dehydrogenase (AADH) are a new class of enzymes that contain a novel heterocyclic *o*-quinone cofactor, TTQ (tryptophan tryptophylquinone), post-translationally derived from two tryptophan residues at the enzyme active site.[1−3] It has a unique heterocyclic





o-quinone skeleton of 6,7-indolequinone with a 2-indolyl group at the 4-position. This cofactor is the redox center of MADH and AADH, catalyzing the oxidation of primary amines to the corresponding aldehydes and ammonia by donating two electrons to a blue copper protein such as amicyanin or azurin (eq 1).[4]

Recently, we have developed a synthetic model (compound **1**) of TTQ cofactor, the molecular geometry, redox potential, and several spectroscopic characteristics of which are very close to those of the native enzymes.[5]



Since TTQ cofactor cannot be isolated intact from the enzyme matrix because of the strong binding through the peptide linkage, such a model study provides valuable information about the physicochemical properties of the cofactor. With regard to the amine-oxidation mechanism by TTQ, a *transamination* mechanism has been clearly demonstrated to operate both in the enzymatic systems and in the model reactions (Scheme 5).[6,7] A similar transamination mechanism has been proposed for quinoprotein amine oxidases that contain another quinone

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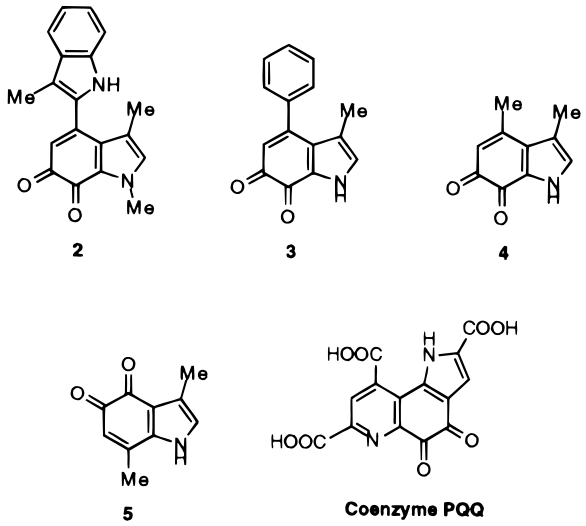
cofactor, TPQ (2,4,5-trihydroxyphenylalanine quinone), in the enzyme active site.[8]

Structural importance of the 3,4-disubstituted 6,7-indolequinone skeleton of cofactor TTQ is another interesting subject. One can assume that the cross linkage between the indolequinone moiety and another tryptophan residue plays an important role in the redox function of TTQ. In this context, the recent X-ray crystal structure determination of the electron-transfer complex of MADH and amicyanin from *Paracoccus denitrificans* indicated that the indole ring at the 4-position (Trp107) is located just between the indolequinone moiety (Trp57) of MADH and the copper ion of amicyanin, suggesting that the indole ring (Trp 107) acts as an electron-transfer pathway from the reduced TTQ to Cu(II) of amicyanin.[4a] On the other hand, TTQ has an active hydrogen atom at the 1-position, deprotonation of which produces a negative charge that can be delocalized into the quinone moiety through conjugation between the pyrrole ring and the quinone moiety. Such an electron delocalization would influence the reactivity of the quinone as in the case of other quinone cofactors PQQ (pyrroloquinolinequinone) and TPQ.[9,10] However, very little is known about the substituent effects on the physicochemical properties and on the chemical reactivities of TTQ. Furthermore, it is very intriguing to know why TTQ has the 6,7-indolequinone framework and whether there is any difference in reactivity between the 6,7-indolequinone derivative and other indolequinones such as the 4,5-quinone isomer. In fact, coenzyme PQQ has a 4,5-indolequinone framework in its structure. In order to address these issues, we have herein synthesized a series of 6,7-indolequinone derivatives (compounds **2**−**4**) and 4,5-indolequinone derivative **5**, and their physicochemical properties and reactivities are compared with those of model compound **1**.[11]

Results and Discussion

Synthesis. 1-Methyl-TTQ model **2** was synthesized starting from the synthetic intermediate **6** of the total synthesis of **1** as shown in Scheme 1.[5] Thus, the indole derivative **6** was first converted into the 1-methylated derivative **7** by MeI in NaH/HMPA system. The carbomethoxy group at the 2-position was then removed by ester hydrolysis followed by thermal decarboxylation using $2CuO{\cdot}Cr_2O_3$ in quinoline at 200 °C. The second indole ring was then constructed by a Fischer indolization on **9** with phenylhydrazine hydrochloride in refluxing ethanol containing a small amount of H_2SO_4. Deprotection of the methoxy group of **10** by trimethylsilyl iodide gave 7-hydroxy derivative **11**, which was finally converted into the 1-methylated indolequinone derivative **2** by Fremy's salt oxidation in aqueous $CH_3CN-KH_2PO_4$.



4-Phenyl- and 4-methyl-substituted 6,7-indolequinone derivatives **3** and **4** were synthesized from 4-phenyl- and 4-methyl-substituted 2-carbomethoxy-3-methyl-7-methoxyindole derivatives **12** and **16**, respectively, by applying the same strategy for the synthesis of **2**: removal of the 2-carbomethoxy group, deprotection of the 7-methoxy substituent to a hydroxy group, and Fremy's salt oxidation to the quinone (Schemes 2 and 3).

4,5-Quinone isomer **5** was also synthesized by basically the same strategy from 2-carbomethoxy-3,7-dimethyl-5-benzyloxyindole (**20**) that can be prepared from 2-methyl-4-benzyloxyaniline and methyl α-ethylacetoacetate according to the established procedure of a combination of a Jaap−Klingemann reaction and a Fischer indolization as in the case of the starting materials **12** and **16**.[12,13] Removal of the carbomethoxy group of **20** by hydrolysis and thermal decarboxylation gave indole derivative **22**, which was converted into the expected 4,5-quinone **5** by the catalytic hydrogenation and the following Fremy's salt oxidation (Scheme 4).

Comparison of Physicochemical Properties. Compound **1** shows a pH-dependent spectrum change between pH 9 and 13 in an aqueous buffer solution containing 10% DMSO, which corresponds to the acid−base equilibrium of 1-NH (the pyrrole proton of the indolequinone).[5b] The absorbance at 434 nm decreases, accompanied by an increase in absorbance at 469 nm with isosbestic points at 361, 436, and 549 nm (λ_{max} = 434 nm, ϵ = 9240 M^{-1} cm^{-1} at pH 6.3, λ_{max} = 469 nm, ϵ = 12100 M^{-1} cm^{-1} at pH 12.9). From the spectral change, the acid-dissociation constant (K_a) of **1** was determined to be 10.6.[5b] Similar spectral changes were obtained in the cases of **3**−**5** (**3**: λ_{max} = 422 nm, ϵ = 7530 M^{-1} cm^{-1} at pH 6.3, λ_{max} = 463 nm, ϵ = 8910 M^{-1} cm^{-1} at pH 13.2; **4**: λ_{max} = 413 nm, ϵ = 3750 M^{-1} cm^{-1} at pH 6.3, λ_{max} = 451 nm, ϵ = 5100 M^{-1} cm^{-1} at pH 12.9; **5**: λ_{max} = 358 nm, ϵ = 2600 M^{-1} cm^{-1} and λ_{max} = 550 nm, ϵ = 1860 M^{-1} cm^{-1}at pH 6.3, λ_{max} = 406 nm, ϵ = 3320 M^{-1} cm^{-1} and λ_{max} = 615 nm, ϵ = 1180 M^{-1} cm^{-1} at pH 12.9) from which K_a values of **3**−**5** were determined to be 10.7, 11.0, and 11.4 by an ordinary least-squares titration curve fitting,[5b] respectively (Table 1). On the other hand, compound **2** did not afford any spectral change within the wide rage of pH values; the λ_{max} = 432 nm is always constant at pH = 1−13,

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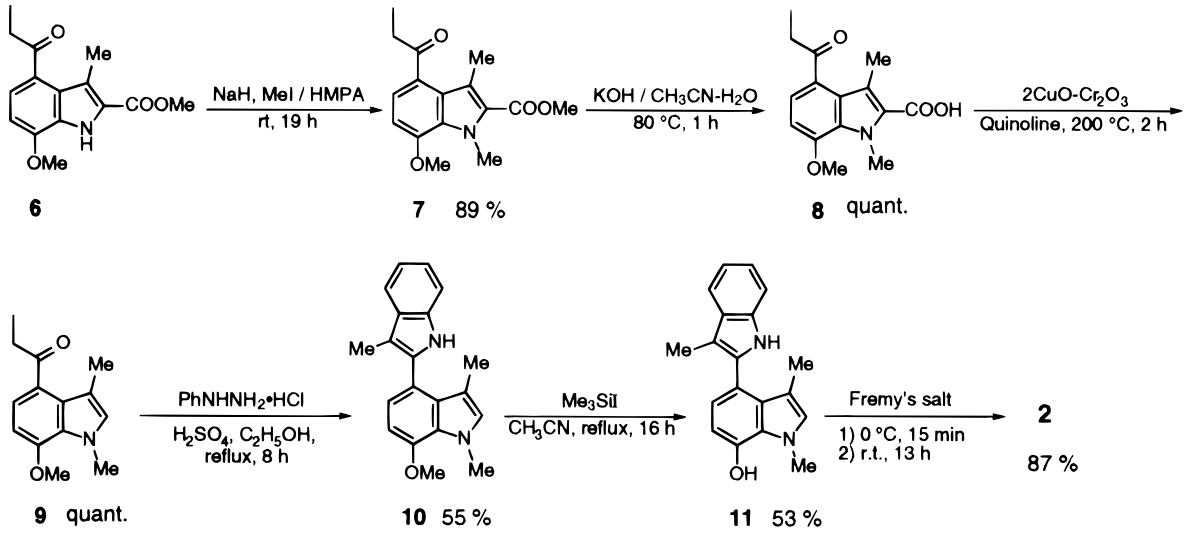
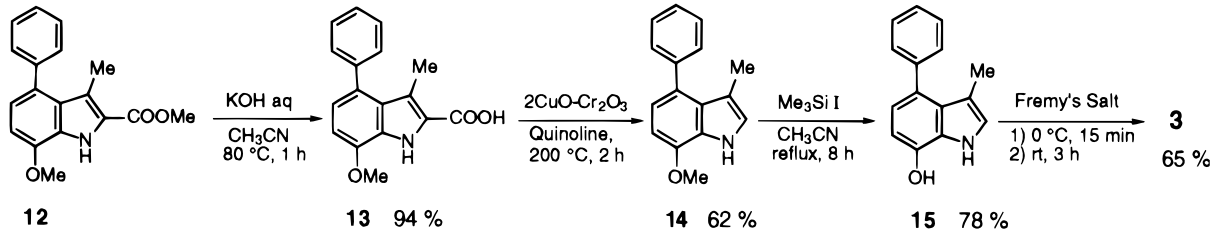
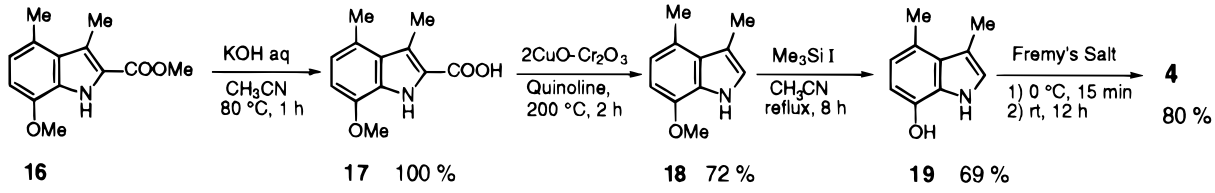
Scheme 1



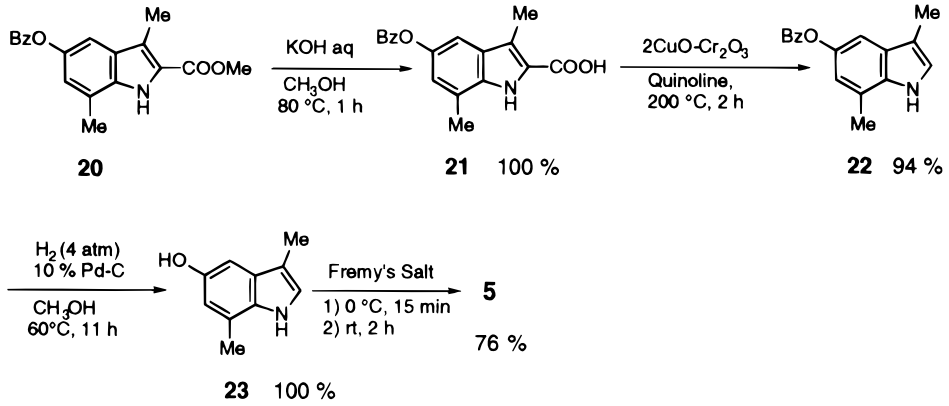
Scheme 2



Scheme 3



Scheme 4



supporting the fact that the pK_a values determined for **1**, **3**, **4**, and **5** correspond to the acid−base equilibrium of the pyrrole proton of the indolequinone ring as we expected. The smaller pK_a values of **1** and **3** as compared to that of **4** indicate that the negative charge generated by the dissociation of the pyrrole proton (1-NH) is stabilized to some extent by the aromatic substituent at the 4-position. Comparison of the pK_a values of **4** and **5** suggests that the conjugative stabilization of the negative charge generated by the dissociation of the pyrrole proton is a little stronger in **4** than in **5**.

UV−vis spectra of the quinones (**2**−**5**) and their quinol forms (the two-electron reduced species) in CH_3CN are shown in Figure 1 (those of **1** were already reported in ref 5b). All the 6,7-indolequinones show a characteristic absorption around 400 nm with a broad shoulder at the longer wavelength, the latter of which can be attributed to the quinonoid n−π* transition.[14] The spectral shape of each quinone is close to that of native TTQ in

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Table 1. Comparison of the Physicochemical Properties of 1−5

		1	2	3	4	5
UV−vis[a]	λ_{max} (nm)	407	412	400	392	499
	ϵ (M^{-1} cm^{-1})	10700	11100	5500	9800	1500
IR quinonoid	$\nu_{C=O}$ (cm^{-1})[b]	1628	1636	1638	1636	1644
^{1}H NMR (δ)[c]	1-H	12.74		12.69	12.50	11.89
	2-H	7.15	7.18	7.10	7.10	6.82
	3-CH_3	1.53	1.48	1.49	2.18	2.18
	5-H	5.86	5.89	5.67	5.73	5.78[d]
^{13}C NMR (ppm)[c]	quinonoid	167.3	167.7	167.0	167.4	174.8
	carbon	183.1	182.3	183.1	183.1	183.3
pK_a of 1-NH[e]		10.6		10.7	11.0	11.4
$E_{1/2}$ (mV vs SCE)[f]		−188	−191	−183	−225	−244

[a] In CH_3CN. [b] KBr disk. [c] In DMSO-d_6. [d] 6-H. [e] In 0.1 M aqueous buffer solution containing 10% DMSO. [f] In 0.1 M phosphate buffer solution containing 30% CH_3CN (pH 7.4).

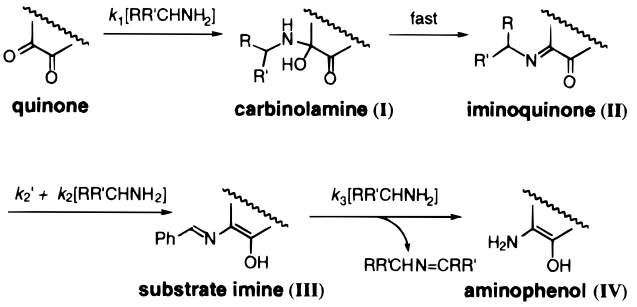
enzymatic systems, although λ_{max} becomes a little smaller in going from **1** to **4**. On the other hand, the 4,5-quinone derivative **5** shows a completely different spectrum as compared to the 6,7-quinone derivatives. Disappearance of the absorption in the visible region by the reduction of the quinone to the quinol form is a similar feature of all the compounds.

Judging from the IR absorption of carbonyl stretching of the quinone ($\nu_{C=O}$) around 1630 cm^{-1} and ^{1}H and ^{13}C NMR chemical shifts of 2-H, 5-H, 6-C, and 7-C, the electronic structure of the 6,7-indolequinone moiety of each 6,7-quinone is essentially the same in the ground state. The lower $\nu_{C=O}$ values (by ca. 50 cm^{-1}) and the lower chemical shifts of 6-C (by ca. 15-20 ppm) as compared to ordinary *o*-quinones[14] are found to be the common features of the 6,7-indolequinones. The lower $\nu_{C=O}$ values in IR and the upfield shift of 1-NH in ^{1}H NMR of the 6,7-indolequinones as compared to those of 4,5-indolequinone **5** may tell us again that the conjugation between the indole ring and the quinone moiety of the 6,7-indolequinone is larger than that of the 4,5-indolequinone, as suggested by the comparison of the pK_a values. A slight downfield shift of 1-NH in going from **5** to **1** indicates that the order of acidity of the pyrrole proton is **1** > **3** > **4** > **5**, being consistent with the order of the pK_a values of the indolequinones.

In the previous study, we assumed that the upfield shift of 3-CH_3 of **1** is attributed to the ring current effect of the indole ring at the 4-position that is twisted about 45° from the quinone ring.[5] Such a conclusion is now confirmed by the fact that the 3-methyl protons of **2** and **3** show essentially the same chemical shift at ca. $\delta = 1.5$ to that of **1**, while the 3-methyl protons of **4** and **5** appear at a normal position ($\delta = 2.18$) as a methyl group connected to an indole ring.[14] Essentially the same chemical shifts of 3-CH_3 of **2** and **3** as compared to that of **1** may also indicate that the dihedral angles of the aromatic groups at the 4-position in **2** and **3** are the same (ca. 45°).

The two-electron redox potentials ($E_{1/2}$) in an aqueous buffer solution containing 10% DMSO (pH 7.4) were determined by cyclic voltammetry. Each quinone shows a reversible redox peak that corresponds to the two-electron quinone/quinol redox couple. As can be seen in Table 1, replacement of the aromatic substituents at the 4-position by the simple alkyl group causes a ca. 40 mV negative shift of $E_{1/2}$, suggesting that the aromatic substituents at the 4-position act as an electron-withdrawing group although such an electronic effect is relatively small. Comparison of $E_{1/2}$ of **4** and **5** indicates that the redox potential of the 6,7-indolequinone is slightly more positive than that of 4,5-indolequinone.

Scheme 5



Reaction with Amines. We have previously demonstrated that the benzylamine oxidation by TTQ model compound **1** proceeds via a *transamination* mechanism through the C-6 iminoquinone and product−imine intermediates (II and III in Scheme 5), which is consistent with the proposed mechanism for the amine−oxidation reaction in the enzymatic systems.[7] In order to examine the substituent effects and the structural importance in the amine−oxidation mechanism, the reactions of the indolequinone derivatives with amines were investigated kinetically under anaerobic conditions.

The iminoquinone formation (from the quinone to II in Scheme 5) with cyclopropylamine [R, R′ = $(-CH_2-)_2$ in Scheme 5] was followed by monitoring the UV−vis spectrum in CH_3OH at 30 °C. A typical spectral change is shown in Figure 2, which was obtained in the reaction of **2** and cyclopropylamine under the pseudo-first-order conditions. As can be seen in Figure 2, a remarkable increase in the absorption band at 394 nm due to the iminoquinone (II) accompanied by a decrease in the absorption band at 420 nm due to the quinone itself is observed with a clear isosbestic point at 410 nm. The increase in absorbance at 394 nm obeys the pseudo-first-order rate law (inset of Figure 2), from which the pseudo-first-order rate constant $k_{obs(1)}$ was obtained. The plot of $k_{obs(1)}$ vs the cyclopropylamine concentration (0−10 mM) gave a straight line passing through the origin without any saturation phenomenon (Figure 3), from the slope of which the second-order rate constant k_1 for the iminoquinone formation step was obtained. Similar spectral changes were obtained in the case of other 6,7-indolequinones and the second-order rate constants k_1, obtained from the slope of the linear plot of $k_{obs(1)}$ vs the amine concentration, are listed in Table 2, together with λ_{max} of the iminoquinone products. The similarity in λ_{max} among all the 6,7-indolequinones suggests that addition position of the amine is always C-6 as confirmed by the NOE measurement and the theoretical calculation on the isolated iminoquinone of **1** with cyclopropylamine.[15]

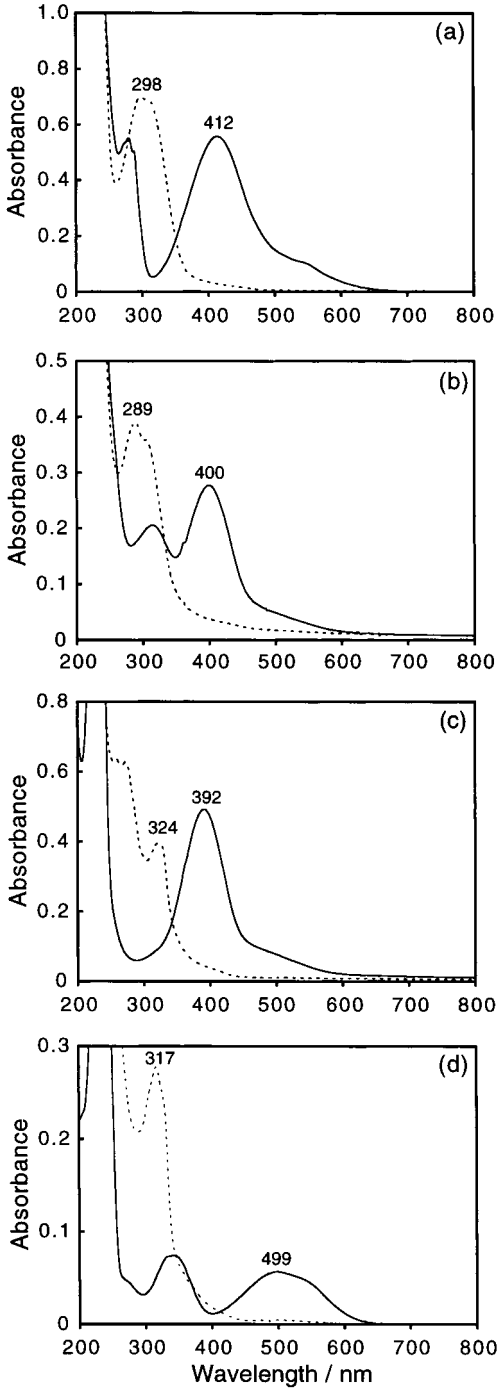


Figure 1. UV−vis spectra of (a) **2** (solid line) and **2H$_2$** (quinol form, dashed line), (b) **3** (solid line) and **3H$_2$** (quinol form, dashed line), (c) **4** (solid line) and **4H$_2$** (quinol form, dashed line), (d) **5** (solid line) and **5H$_2$** (quinol form, dashed line) in CH_3CN at a concentration of 5.0×10^{-5} M. The reduced forms were generated in situ by the reduction with CH_3NHNH_2 (10 equiv).

A spectral change due to the iminoquinone formation of 4,5-indolequinone **5** with cyclopropylamine is shown in Figure 4. In this case as well, a remarkable increase in the absorption band at 468 nm due to the iminoquinone accompanied by a decrease at 534 nm due to the quinone itself is observed with a clear isosbestic point at 512 nm, and the second-order rate constant k_1 for the

(15) In the previous study,[7a] we have demonstrated that the amine-adduct formation occurs at the 6-position of **1** based on NOE on the isolated products and theoretical calculations.

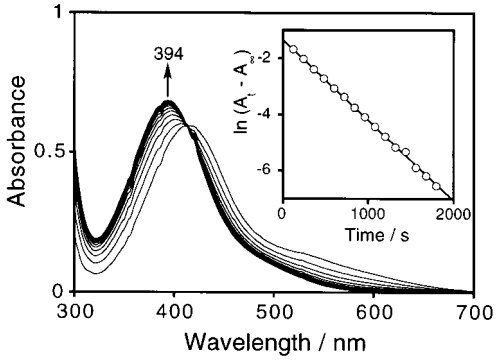


Figure 2. Spectral change observed upon addition of cyclopropylamine (10 mM) to a CH_3OH solution of **2** (5.0×10^{-5} M) at 30 °C under anaerobic conditions; interval, 120 s. Inset: The pseudo-first-order plot.

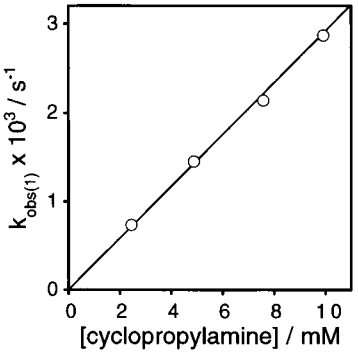


Figure 3. Dependence of $k_{obs(1)}$ on the cyclopropylamine concentration for the reaction of **2** (5.0×10^{-5} M) with cyclopropylamine CH_3OH at 30 °C under anaerobic conditions.

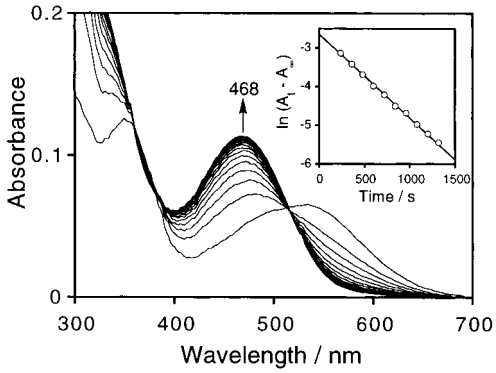


Figure 4. Spectral change observed upon addition of cyclopropylamine (10 mM) to a CH_3OH solution of **5** (5.0×10^{-5} M) at 30 °C under anaerobic conditions; interval, 120 s. Inset: The pseudo-first-order plot.

iminoquinone formation is obtained similarly from the slope of the linear plot of $k_{obs(1)}$ vs the amine concentration.

As can be easily recognized from Table 2, there is no significant difference in k_1 among the 6,7-indolequinones, suggesting that the electronic effect of the substituents on the iminoquinone formation is rather small, if any. Reactivity of the 6,7-indolequinones and the 4,5-quinone derivative is almost the same. It should be noted, however, that the small difference in k_1 between **1** and **2** may be good evidence for the regioselective amine−adduct formation at the 6-position of the quinone ring. If the amine-adduct formation occurred at the 7-position, k_1 for **2** would be much smaller than that of **1** because of the steric hindrance by the methyl group at the 1-position of **2**.

Table 2. Second-Order Rate Constants (k_1) for the Substrate−Imine Formation with Cyclopropylamine and λ_{max} of the Iminoquinone Derivatives[a]

quinone	λ_{max}/nm	k_1/M^{-1} s^{-1}
1	392[b]	0.20[b]
2	394	0.28
3	388	0.24
4	380	0.15
5	468	0.26

[a] [Quinone] = 5.0×10^{-5} M, in CH_3OH, at 30 °C, under N_2. [b] Taken from ref 7a.

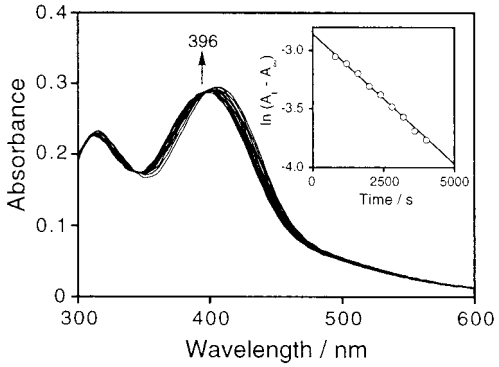


Figure 5. Spectral change observed upon addition of benzylamine (1 mM) to a CH_3OH solution of **3** (5.0×10^{-5} M) at 30 °C under anaerobic conditions; interval, 600 s. Inset: The pseudo-first-order plot.

Table 3. Rate Constants k_1, k_2, and k_3 in the Reactions of the Quinones with Benzylamine[a]

quinone	k_1/M^{-1} s^{-1}	k_2/M^{-1} s^{-1}	k_3/M^{-1} s^{-1}
1	0.27[b]	0.10[b]	0.014[b]
2	0.36	0.087	0.024
3	0.24	0.10	0.033
4	*c*	0.067	*c*
5	0.37	0.054	*c*

[a] [Quinone] = 5.0×10^{-5} M, in CH_3OH, at 30 °C, under N_2. [b] Taken from ref 7a. [c] Could not be determined accurately.

The reactions of the *o*-quinones with benzylamine (R = H, R′ = Ph in Scheme 5) were also investigated in CH_3OH. At low concentrations of benzylamine (below 1.5 mM), formation of the iminoquinone derivative is observed predominantly as has been already demonstrated using model compound **1**.[7a] A typical spectral change is presented in Figure 5, which was obtained in the reaction of **3** and benzylamine. The k_1 value for the iminoquinone formation was determined as 0.24 M^{-1} s^{-1} from the slope of the linear plot of $k_{obs(1)}$ vs [benzylamine] as in the case of cyclopropylamine. The k_1 values for the formation of the iminoquinone intermediates of **2** and **5** were also determined in a similar manner, and they are listed in Table 3 (k_1 for **4** could not be determined accurately because of the overlap of the spectral change by the following reaction). The difference in the k_1 values for the iminoquinone formation between the *o*-quinone and benzylamine is as small as in the case of cyclopropylamine.

At higher concentrations of benzylamine (10−20 mM), however, the reaction proceeded further to cause the rearrangement of the iminoquinone to the product−imine intermediate (II to III in Scheme 5). Figure 6 shows the spectral change observed upon addition of benzylamine (10 mM) to a CH_3OH solution of **3** (5.0×10^{-5} M) at 30 °C under anaerobic conditions. During the course of the reaction, the absorption due to the quinone (spectrum a)

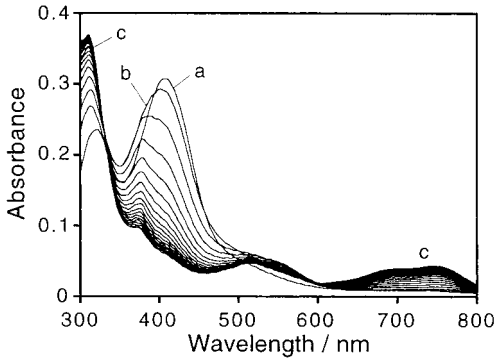


Figure 6. Spectral change observed upon addition of benzylamine (10 mM) to a CH_3OH solution of **3** (5.0×10^{-5} M) at 30 °C under anaerobic conditions; interval, 120 s.

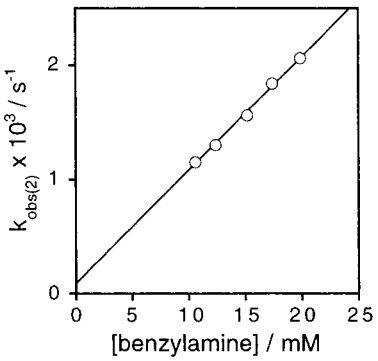


Figure 7. Dependence of $k_{obs(2)}$ on the benzylamine concentration for the reaction of **3** (5.0×10^{-5} M) with benzylamine in CH_3OH at 30 °C under anaerobic conditions.

readily moved to that of the iminoquinone (after a few minutes, spectrum b), which then decreased gradually (for ca. 30 min). The absorption around 380 nm decreased further at the prolonged reaction time, and a new absorption band at 310 nm slowly developed (from ca. 30 min to a few hours, spectrum c in Figure 6). As has been already demonstrated using compound **1**, the first rapid change in the UV−vis spectrum corresponds to the iminoquinone formation for which the rate constant (k_1) was already determined as listed in Table 3. The second one is attributed to the rearrangement of the iminoquinone to the product−imine (II to III in Scheme 5), and the third step correspond to the imine-exchange reaction of the product−imine to produce the aminophenol product (IV in Scheme 5) and *N*-benzylidenebenzylamine (Ph-CH_2N=CHPh, R = Ph amd R′ = H in Scheme 5). The observed rate constants, $k_{obs(2)}$ and $k_{obs(3)}$, for the second and the third steps were determined by the computer curve fitting with the corresponding exponential terms, respectively. The plot of $k_{obs(2)}$ vs [amine] gives a straight line (Figure 7) from which k_2 (the rate constant for the general base-catalyzed rearrangement from II to III) was obtained from the slope as 0.10 M^{-1} s^{-1}.[16] The k_2 values for other quinones were determined in a similar manner, and they are also listed in Table 3.

(16) The previous study using **1**[7a] has demonstrated that the rearrangement step of the substrate−imine to the product−imine intermediate consists of the spontaneous (noncatalyzed) rearrangement and the general base-catalyzed one by the added amine; $k_{obs(2)} = k_2' + k_2$[amine]. Since k_2' corresponds to the intercept of the linear plot of $k_{obs(2)}$ vs [amine], k_2' involves large experimental errors. Thus, in this study, only k_2 (the slope of the linear plot) is used to compare the reactivity in the rearrangement process.

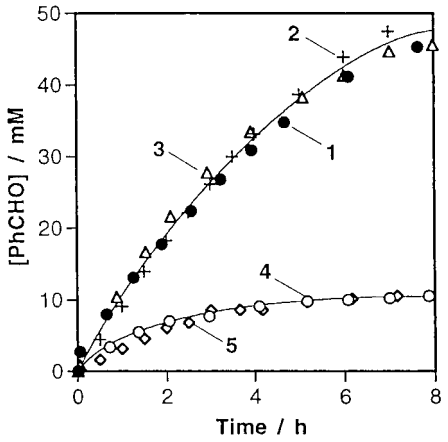
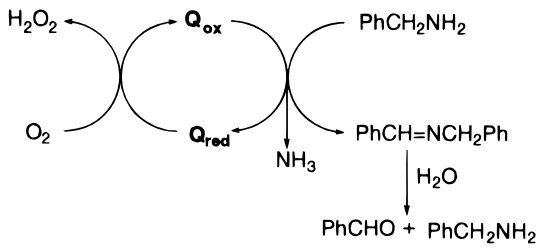


Figure 8. Time courses of the oxidation of benzylamine (100 mM) catalyzed by the *o*-quinones (**1**−**5**; 1 mM) in CH_3OH under aerobic conditions. The formation of benzaldehyde was monitored by HPLC (see Experimental Section).

Scheme 6



The rate constants k_3 for formation of the aminophenol product (IV in Scheme 5) from the product−imine were determined from the linear dependence of the observed pseudo-first-order rate constant $k_{obs(3)}$ on the benzylamine concentration (see Table 3). The k_3 value for the aminolysis in case of **4** and **5**, however, could not determined by this method, since the spectra of the product−imines and the corresponding aminophenols are almost identical. As in the case of iminoquinone formation step, reactivities in the product−imine formation and the aminophenol formation steps were not so different among the *o*-quinone derivatives investigated here.

Compound **1** has been shown to catalyze aerobic oxidation of benzylamine to benzaldehyde in CH_3OH (Scheme 6).[5,7] The catalytic efficiency of **1** was found to be much higher than that of ordinary *o*-quinones such as phenanthrenequinone, 1,7-phenanthrolinequinone, 1,2-naphthoquinone, 1,4-benzoquinone, and so on.[7a] In order to know the catalytic efficiency of the *o*-indolequinone derivatives in the aerobic benzylamine oxidation, the reaction of the *o*-indolequinones (1 mM) and benzylamine (100 mM) was examined in CH_3OH under O_2 atmosphere at room temperature.

Figure 8 shows the time course of the catalytic reaction that was monitored by following the formation of benzaldehyde by HPLC. The maximum amount of benzaldehyde formed is 50 mM, since the primary product $PhCH_2N{=}CHPh$ formed from PhCH=X (X = O or NH) and $PhCH_2NH_2$ is spontaneously converted into PhCHO and $PhCH_2NH_2$ by hydrolysis in the HPLC column (Scheme 6). Thus, the oxidation of benzylamine by **1** proceeded until all of the substrate was consumed. As can be seen in Figure 8, compounds **2** and **3** show essentially the same high catalytic activity as compound **1**, but on the other hand, compounds **4** and **5** are deactivated in an early stage of the reaction. In the anaerobic single-turnover reaction, it has been demonstrated that the reactivities of all the quinones are almost the same in each step. Furthermore, it has been confirmed that the reoxidation of the reduced quinone (**1H$_2$**) by O_2 is much faster than the reaction of **1** with benzylamine under the basic conditions employed.[7a] Since the redox potentials of **2**−**5** are more negative than that of **1** (see Table 1), reoxidation of the reduced species of **2**−**5** by O_2 must be faster than that of **1**, suggesting that the reoxidation step of the aminophenol by O_2 is not rate determining in the catalytic reaction (Scheme 6). From these considerations, we assume that the lower catalytic activity of the *o*-quinones having the methyl group at the 4-position (**4** and **5**) as compared to that of the *o*-quinones having the aromatic substituent at the 4-position (**1**−**3**) may be attributed to the less stability of **4** and **5** under the reaction conditions. A possible pathway in the deactivation of the catalyst is a Michael-type addition of the amine substrate to the C-4 position of the quinone ring. Relatively bulky aromatic groups at C-4 in **1**−**3** may protect the quinone ring from such an undesirable addition reaction at C-4 by the amine.[17]

Concluding Remarks. We have synthesized a series of 3,4-disubstituted 6,7-indolequinones (**2**−**4**) and 3,7-disubstituted 4,5-indolequinone (**5**) as the model compounds for the novel organic cofactor TTQ of bacterial amine dehydrogenases. The substituent and structural effects on the physicochemical properties of the quinones have been discussed in detail by comparing the spectroscopic data (UV−vis, IR, ^{1}H- and ^{13}C-NMR), pK_a values of the pyrrole proton, and the two-electron redox potentials with those of model compound **1**. Reactivity of each quinone in the transamination process has been also investigated kinetically. Although the substituent and the structural effects on the physicochemical properties and the chemical reactivity of the indolequinone derivatives are rather small, some interesting points can be withdrawn from the present model study as follows.

In the case of PQQ and TPQ cofactors, the active hydrogen (pyrrole proton 1-NH of PQQ and hydroxyl proton 4-OH of TPQ) has been shown to play an essential role to enhance the reactivity of the cofactor in the amine oxidation reaction.[9,10] Replacement of the active hydrogens of PQQ and TPQ model compounds by a methyl group drastically depresses the reactivity of the quinones toward amines. In the case of TTQ, however, replacement of the active hydrogen at the 1-position by a methyl group does not affect the reactivity of the indolequinone. The crystal structure of methylamine dehydrogenase reveals that the pyrrole proton of the indolequinone is hydrogen bonded to a backbone carbonyl and likely inaccessible to solvent or an external base,[2] suggesting that dissociation of the pyrrole proton of TTQ is not required for the amine oxidation reaction in the enzymatic system. Such a contemplation from the structural information of the enzyme is consistent with the result of the present model study, demonstrating that **1** and **2** have essentially the same reactivity in the amine-oxidation reaction.

The crystal structure of the electron-transfer complex of MADH and amicyanin from *P. denitrificans* indicated that the indole ring at the 4-position (Trp107) plays an

(17) In contrast to the case of compound **1**, which provides the aminophenol as a sole isolable product in the reaction with benzylamine,[7] the corresponding aminophenol derivatives were not obtained from the reactions of **4** and **5** with benzylamine on a preparative scale.

important role for electron transfer from the reduced TTQ to Cu(II) of amicyanin.[4a] In this context, one can assume that the enzyme controls the electron-transfer rate by adjusting the dihedral angle between the two indole rings.[18] The difference of the redox potential between **1** and **4** clearly indicates that the conjugation between the indolequinone ring and another indole ring increases the redox potential of the quinone. Thus, it may be possible to control the redox potential of the indolequinone by tuning the dihedral angle of the TTQ molecule, although quantitative estimation of such a function is difficult in our model system because of the flexibility of the rotation around the single bond between the two aromatic groups in solution.

With regard to the structural importance of cofactor TTQ, the present result shows that the 6,7-indolequinone skeleton is not necessarily required for the amine-oxidation reaction. In fact, coenzyme PQQ, which has a 4,5-indolequinone framework, acts as a better catalyst for the benzylamine oxidation in our model reaction.[7,9] We think that the 6,7-indolequinone skeleton of TTQ is derived by geometrical restriction of the two tryptophans (precursors of TTQ) at the active site of the apo-enzyme. In other words, only the 6- and 7-positions of the indole ring of Trp57 may be accessible to solvent during the biosynthetic process.

Experimental Section

All the chemicals used in this study except for the model compounds and their synthetic intermediates were commercial products of the highest available purity and were further purified by standard methods, if necessary.[19] Melting points are uncorrected. Mass spectra were determined at an ionization voltage of 70 eV. ^{1}H NMR and ^{13}C NMR spectra were recorded at 270 MHz using $CDCl_3$ or DMSO-d_6 as a solvent and TMS as an internal reference. UV−vis spectra were taken with a photodiode array spectrometer (Hewlett-Packard HP8452A). The cyclic voltammetry measurements were performed with a three-electrode system consisting of a glassy carbon working electrode, a platinum plate auxiliary electrode, and an SCE reference electrode. The glassy carbon electrode was polished with 0.05-mm alumina powder, sonicated to remove the powder, and washed with water. All electrochemical measurements were carried out at 25 °C under an atmospheric pressure of nitrogen.

Synthesis. 1,3-Dimethyl-2-carbomethoxy-4-propionyl-7-methoxyindole (7). 2-Carbomethoxy-3-methyl-4-propionyl-7-methoxyindole (**6**) was prepared according to the reported procedure.[5] An HMPA (60 mL) solution of **6** (1.50 g, 5.45 mmol) was added dropwise to NaH (218 mg, 5.45 mmol, 60% content mineral oil suspension that was washed with dry *n*-hexane three times before use) at 0 °C under N_2, and the mixture was stirred at room temperature for 3 h. MeI (339 μL, 5.45 mmol) was then added to the mixture, which was stirred at room temperature for an additional 19 h. After that, the reaction was quenched by adding H_2O (200 mL), and the product was extracted with ether three times (100 mL × 3). The combined organic layer was washed with H_2O and dried over $MgSO_4$. After removal of $MgSO_4$ by filtration, the product was obtained in an 89% yield by evaporation of the solvent and washing the remained solid with ether several times: mp 85−87 °C; IR (KBr) 1710 and 1670 cm^{-1} (C=O); ^{1}H NMR ($CDCl_3$) δ 1.25 (3 H, t, J = 7.3 Hz), 2.38 (3 H, s), 2.97 (2 H, q, J = 7.3 Hz), 3.94 (3 H, s), 3.96 (3 H, s), 4.23 (3 H, s), 6.65 (1 H, d, J = 8.1 Hz), 7.23 (1 H, d, J = 8.1 Hz); MS (EI) m/z 289 (M^+). Anal. Calcd for $C_{16}H_{19}NO_4$: C, 66.42; H, 6.62; N, 4.84. Found: C, 66.25; H, 6.56; N, 4.82.

1,3-Dimethyl-2-carboxy-4-propionyl-7-methoxyindole (8). An indole derivative **7** (1.40 g, 4.84 mmol) was treated in 1 *N* KOH aqueous CH_3CN solution (80 mL, 1:1, v/v) at 80 °C for 1 h. Acidification of the reaction mixture to pH 3 with 1 *N* HCl gave a white precipitate that was isolated by centrifugation and dried *in vacuo* (100%): mp 189−191 °C; IR (KBr) 1676 cm^{-1} (C=O); ^{1}H NMR ($CDCl_3$) δ 1.26 (3 H, t, J = 7.3 Hz), 2.48 (3 H, s), 2.98 (2 H, q, J = 7.3 Hz), 3.98 (3 H, s), 4.30 (3 H, s), 6.68 (1 H, d, J = 8.1 Hz), 7.24 (1 H, d, J = 8.1 Hz); HRMS (EI) m/z 275.1158 (M^+), calcd for $C_{15}H_{17}NO_4$ 275.1157.[20]

1,3-Dimethyl-4-propionyl-7-methoxyindole (9). A mixture of **8** (25.0 mg, 0.091 mmol) and $2CuO-Cr_2O_3$ (3.39 mg) in quinoline (1.2 mL) was heated at 200 °C for 2 h. The mixture was then extracted with ether and washed with 1 *N* HCl and saturated $NaHCO_3$(aq). The organic layer was further washed with 1 *N* HCl several times and dried over $MgSO_4$. After removal of $MgSO_4$ by filtration, evaporation of the solvent gave pale brown solids **9** quantitatively: mp 112−113 °C; IR (KBr) 1674 cm^{-1} (C=O); ^{1}H NMR ($CDCl_3$) δ 1.24 (3 H, t, J = 7.3 Hz), 2.25 (3 H, d, J = 1.1 Hz), 2.99 (2 H, q, J = 7.3 Hz), 3.94 (3 H, s), 3.98 (3 H, s), 6.54 (1 H, d, J = 8.1 Hz), 6.78 (1 H, br s), 7.35 (1 H, d, J = 8.1 Hz); MS (EI) m/z 231 (M^+). Anal. Calcd for $C_{14}H_{17}NO_2$: C, 72.70; H, 7.41; N, 6.06. Found: C, 72.35; H, 7.26; N, 5.99 (a sample for the elemental analysis was obtained by washing the solids with ether several times).

1,3-Dimethyl-4-(3′-methylindol-2′-yl)-7-methoxyindole (10). An ethanol (20 mL) solution of **9** (1.00 g, 4.32 mmol) and phenylhydrazine hydrochloride (0.94 g, 6.49 mmol) containing concentrated H_2SO_4 (2.5 mL) was stirred at the refluxing temperature for 8 h. The reaction mixture was diluted with water, extracted with $CHCl_3$, and dried over $MgSO_4$. After removal of $MgSO_4$ by filtration, evaporation of the solvent gave yellow solids from which **10** was isolated as a white solid in a 55% yield by flash column chromatography (SiO_2, $CHCl_3$): mp 114−116 °C; IR (KBr) 3416 cm^{-1} (NH); ^{1}H NMR ($CDCl_3$) δ 1.75 (3 H, s), 2.20 (3 H, s), 3.94 (3 H, s), 4.01 (3 H, s), 6.63 (1 H, d, J = 8.1 Hz), 6.68 (1 H, s), 6.93 (1 H, d, J = 8.1 Hz), 7.13−7.18 (2 H, m), 7.30 (1 H, d, J = 6.8 Hz), 7.59 (1 H, d, J = 6.5 Hz), 7.87 (1 H, br s); MS (EI) m/z 304 (M^+).[20]

1,3-Dimethyl-4-(3′-methylindol-2′-yl)-7-hydroxyindole (11). A CH_3CN (10 mL) solution of **10** (723 mg, 2.38 mmol) and trimethylsilyl iodide (3.4 mL, 23.8 mmol) was stirred at the refluxing temperature for 16 h. The reaction was quenched by adding $Na_2S_2O_3$(aq) and was extracted with CH_2Cl_2 three times. The combined organic layer was washed with aqueous $Na_2S_2O_3$ and H_2O and then dried over $MgSO_4$. After removal of $MgSO_4$ by filtration, evaporation of the solvent gave a crude product that was purified by flash column chromatography (SiO_2, $CHCl_3$) (53% yield): mp 75−78 °C; IR (KBr) 3428 (NH), 3200−3550 cm^{-1} (OH); ^{1}H NMR ($CDCl_3$) δ 1.77 (3 H, s), 2.20 (3 H, s), 4.06 (3 H, s), 4.91 (1 H, br s), 6.51 (1 H, d, J = 7.6 Hz), 6.73 (1 H, s), 6.85 (1 H, d, J = 7.6 Hz), 7.12−7.23 (2 H, m), 7.33 (1 H, d, J = 6.6 Hz), 7.60 (1 H, d, J = 6.6 Hz), 7.93 (1 H, br s); ^{13}C NMR ($CDCl_3$) 8.6, 10.0, 60.1, 105.8, 109.0, 109.9, 110.3, 117.9, 118.1, 118.6, 120.8, 121.8, 125.5, 128.6, 129.0, 129.6, 133.7, 134.6, 142.8 ppm (16 sp^2 carbons); MS (EI) m/z 290 (M^+).[20]

1,3-Dimethyl-4-(3′-methylindol-2′-yl)indole-6,7-dione (2). To a CH_3CN solution (60 mL) of **11** (365 mg, 1.26 mmol) was added Fremy's salt (1.35 g, 5.01 mmol) in 0.062 *M* KH_2PO_4 aqueous solution (60 mL) at 0 °C. The mixture was stirred at 0 °C for 15 min and at room temperature for 13 h. The final reaction mixture was then extracted with AcOEt. After the extract was dried over K_2CO_3, evaporation of the solvent gave a dark brown residue from which quinone **2** was isolated in a 87% yield by flash column chromatography (SiO_2, $CHCl_3$, for the elemental analysis, the sample was washed with ether

(18) Brooks, H. B.; Davidson, V. L. *J. Am. Chem. Soc.* **1994**, *116*, 11201.

(19) Perrin, D. D.; Armarego, W. L. F.; Perrin, D. R. *Purification of LaboratoryChemicals*; Pergamon Press: Elmsford, NY, 1966.

(20) In spite of our efforts, a satisfactory result of elemental analysis could not be obtained. It is probably due to the hygroscopicity by the hydroxyl or the carboxyl group in the molecule. In order to show the purity of the sample, a copy of the ^{1}H NMR spectrum is provided as Supporting Information.

several times): mp >300 °C; IR (KBr) 3280 (NH), 1636 and 1614 cm^{-1} (C=O); ^1H NMR (DMSO-d_6) δ 1.48 (3 H, s), 2.29 (3 H, s), 3.88 (3 H, s), 5.89 (1 H, s), 7.04 (1 H, t, J = 7.4 Hz), 7.15 (1 H, t, J = 7.4 Hz), 7.18 (1 H, s), 7.35 (1 H, d, J = 8.1 Hz), 7.56 (1 H, d, J = 8.1 Hz), 11.23 (1 H, br s); ^{13}C NMR (DMSO-d_6) 9.2, 10.3, 36.4, 109.7, 111.4, 118.9, 119.2, 120.1, 122.6, 123.6, 127.1, 127.6, 128.2, 130.0, 135.2, 136.2, 143.9 (14 sp^2 carbons), 167.7 (C=O), 182.3 (C=O) ppm; MS (EI) m/z 304 (M^+), 306 (M^+ + 2, characteristic peak for *o*-quinone compounds[14]). Anal. Calcd for $C_{19}H_{16}N_2O_2$: C, 74.98; H, 5.30; N, 9.20. Found: C, 74.68; H, 5.27; N, 9.08.

2-Carboxy-3-methyl-4-phenyl-7-methoxyindole (13). The starting indole derivative (**12**) was prepared from 4-methoxy-3-biphenylamine and methyl α-ethylacetoacetate according to the established procedure (combination of a Jaap−Klingemann reaction and a Fischer indolization).[12,13] Compound **12** (65.5 mg, 0.222 mmol) was treated in 1 *N* KOH aqueous CH_3CN solution (7 mL, 1:1, v/v) at 80 °C for 1 h. Acidification of the reaction mixture to pH 3 with 1 *N* HCl gave a white precipitate that was isolated by centrifugation and dried *in vacuo* (94%): mp >300 °C; IR (KBr) 3424 (NH), 1658 cm^{-1} (C=O); ^1H NMR ($CDCl_3$) δ 2.18 (3 H, s), 2.69 (1 H, br s), 4.01 (3 H, s), 6.76 (1 H, d, J = 7.8 Hz), 6.89 (1 H, d, J = 7.8 Hz), 7.39−7.47 (5 H, m), 9.04 (1 H, br s); HRMS (EI) m/z 281.1064 (M^+), calcd for $C_{17}H_{15}O_3N$ 281.1053.[20]

3-Methyl-4-phenyl-7-methoxyindole (14). A mixture of **13** (390 mg, 1.39 mmol) and $2CuO-CrO_2O_3$ (51.8 mg) in quinoline (17 mL) was heated at 200 °C for 2 h. The mixture was then extracted with ether and washed with 1 *N* HCl and saturated $NaHCO_3$(aq). The organic layer was further washed with 1 *N* HCl several times and dried over $MgSO_4$. After removal of $MgSO_4$ by filtration, evaporation of the solvent gave white solids from which pure product **14** was obtained by washing with ether in a 62% yield: mp 52−57 °C; IR (KBr) 3436 cm^{-1} (NH); ^1H NMR ($CDCl_3$) δ 1.87 (3 H, s), 3.99 (3 H, s), 6.67 (1 H, d, J = 7.8 Hz), 6.89 (1 H, d, J = 7.8 Hz), 6.95 (1 H, s), 7.36−7.42 (5 H, m), 8.22 (1 H, br s); MS (EI) m/z 237 (M^+). Anal. Calcd for $C_{16}H_{15}NO$: C, 80.98; H, 6.37; N, 5.90. Found: C, 80.89; H, 6.35; N, 5.83.

3-Methyl-4-phenyl-7-hydroxyindole (15). A CH_3CN (20 mL) solution of **14** (338 mg, 1.42 mmol) and trimethylsilyl iodide (2.0 mL, 14.0 mmol) was stirred at the refluxing temperature for 8 h. The reaction was quenched by adding $Na_2S_2O_3$(aq) and was extracted with CH_2Cl_2 three times. The combined organic layer was washed with aqueous $Na_2S_2O_3$ and H_2O and then dried over $MgSO_4$. After removal of $MgSO_4$ by filtration, evaporation of the solvent gave a crude product that was purified by flash column chromatography (SiO_2, $CHCl_3$) (78% yield): oily material; IR (KBr) 3444 (NH), 3200−3500 cm^{-1} (OH); ^1H NMR ($CDCl_3$) δ 1.86 (3 H, s), 4.89 (1 H, br s), 6.58 (1 H, d, J = 7.6 Hz), 6.79 (1 H, d, J = 7.6 Hz), 6.96 (1 H, s), 7.33−7.43 (5 H, m), 8.22 (1 H, br s); MS (EI) m/z 223 (M^+).[20]

3-Methyl-4-phenylindole-6,7-dione (3). To a CH_3CN solution (70 mL) of **15** (246 mg, 1.10 mmol) was added Fremy's salt (1.18 g, 4.40 mmol) in 0.062 M KH_2PO_4 aqueous solution (70 mL) at 0 °C. The mixture was stirred at 0 °C for 15 min and at room temperature for 3 h. The final reaction mixture was then extracted with AcOEt. After the extract was dried over $MgSO_4$, evaporation of the solvent gave a dark brown residue from which quinone **3** was isolated in 65% yield by flash column chromatography (SiO_2, $CHCl_3$, for the elemental analysis, the sample was washed with ether several times): mp >300 °C; IR (KBr) 3288 (NH), 1638 cm^{-1} (C=O); ^1H NMR (DMSO-d_6) δ 1.49 (3 H, s), 5.67 (1 H, s), 7.10 (1 H, s), 7.30−7.50 (5 H, m), 12.69 (1 H, br s); ^{13}C NMR (DMSO-d_6) 11.6, 120.7, 122.4, 126.9, 128.2, 128.6, 129.1, 130.3, 130.5, 137.3, 153.4 (10 sp^2 carbons), 167.0 (C=O), 183.1 (C=O) ppm; MS (EI) m/z 239 (M^+ + 2, characteristic peak for *o*-quinone compounds[14]), HRMS m/z 237.0779, calcd for $C_{15}H_{11}NO_2$ 237.0790. Anal. Calcd for $C_{15}H_{11}NO_2$: C, 75.94; H, 4.67; N, 5.90. Found: C, 75.66; H, 4.58; N, 5.80.

2-Carboxy-3,4-dimethyl-7-methoxyindole (17). The starting material **16** was prepared from 2-methoxy-5-methylaniline and methyl α-ethylacetoacetate according to the established procedure (combination of a Jaap−Klingemann reaction and a Fischer indolization).[12,13] The indole derivative **16** (93.6 mg, 0.402 mmol) was treated in 1 *N* KOH aqueous CH_3CN solution (13 mL, 1:1, v/v) at 80 °C for 1 h. Acidification of the reaction mixture to pH 3 with 1 *N* HCl gave a white precipitate that was isolated by centrifugation and dried *in vacuo* (100%): mp 224−226 °C; IR (KBr) 3429 (NH), 2940 (−COOH), 1690 cm^{-1} (C=O); ^1H NMR ($CDCl_3$) δ 2.67 (3 H, d, J = 1.1 Hz), 2.85 (3 H, s), 3.94 (3 H, s), 6.58 (1 H, d, J = 7.6 Hz), 6.73 (1 H, dd, J = 1.1 and 7.6 Hz), 8.88 (1 H, br s); MS (EI) m/z 219 (M^+).[20]

3,4-Dimethyl-7-methoxyindole (18). A mixture of **17** (87.8 mg, 0.40 mmol) and $2CuO-Cr_2O_3$ (18.4 mg, 0.059) in quinoline (6 mL) was heated at 200 °C for 2 h. The mixture was then extracted with ether and washed with 1 *N* HCl and saturated $NaHCO_3$(aq). The organic layer was further washed with 1 *N* HCl several times and dried over $MgSO_4$. After removal of $MgSO_4$ by filtration, evaporation of the solvent gave a crude product from which **18** was isolated by flash column chromatography (SiO_2, $CHCl_3$) in a 72% yield: mp 75−77 °C; IR (KBr) 3440 cm^{-1} (NH); ^1H NMR ($CDCl_3$) δ 2.49 (3 H, d, J = 0.8 Hz), 2.65 (3 H, s), 3.92 (3 H, s), 6.49 (1 H, d, J = 7.8 Hz), 6.71 (1 H, dd, J = 0.8 and 7.8 Hz), 6.89 (1 H, d, J = 1.1 Hz), 8.06 (1 H, br s); MS (EI) m/z 175 (M^+). Anal. Calcd for $C_{11}H_{13}NO$: C, 75.40; H, 7.48; N, 7.99. Found: C, 75.25; H, 7.39; N, 7.89.

3,4-Dimethyl-7-hydroxyindole (19). A CH_3CN (8 mL) solution of **18** (98.0 mg, 0.56 mmol) and trimethylsilyl iodide (1.12 mg, 5.6 mmol) was stirred at the refluxing temperature for 8 h. The reaction was quenched by adding $Na_2S_2O_3$(aq) and extracted with CH_2Cl_2 three times. The combined organic layer was washed with aqueous $Na_2S_2O_3$ and H_2O and then dried over $MgSO_4$. After removal of $MgSO_4$ by filtration, evaporation of the solvent gave a crude product that was purified by flash column chromatography (SiO_2, $CHCl_3$) (69% yield): mp 120−123 °C; IR (KBr) 3448 (NH and OH); ^1H NMR ($CDCl_3$) δ 2.49 (3 H, d, J = 0.8 Hz), 2.64 (3 H, s), 4.68 (1 H, br s), 6.43 (1 H, d, J = 7.8 Hz), 6.61 (1 H, dd, J = 0.8 and 7.8 Hz), 6.91 (1 H, d, J = 1.1 Hz), 8.02 (1 H, br s); MS (EI) m/z 161 (M^+).[20]

3,4-Dimethylindole-6,7-dione (4). To a CH_3CN solution (24 mL) of **19** (62.0 mg, 0.385 mmol) was added Fremy's salt (418.3 mg, 1.56 mmol) in 0.062 *M* KH_2PO_4 aqueous solution (24 mL) at 0 °C. The mixture was stirred at 0 °C for 15 min and at room temperature for 12 h. The final reaction mixture was then extracted with CH_2Cl_2. After the extract was dried over $MgSO_4$, evaporation of the solvent gave a dark brown residue from which quinone **4** was isolated in 80% yield by flash column chromatography (SiO_2, $CHCl_3$, for the elemental analysis, the sample was washed with ether several times): mp >300 °C; IR (KBr) 3296 (NH), 1636 cm^{-1} (C=O); ^1H NMR (DMSO-d_6) δ 2.18 (3 H, s), 2.26 (3 H, d, J = 1.4 Hz), 5.73 (1 H, d, J = 1.4 Hz), 7.10 (1 H, d, J = 2.4 Hz), 12.5 (1 H, br s); ^{13}C NMR (DMSO-d_6) 11.9, 21.2, 120.6, 121.6, 128.0, 130.0, 152.3 (5 sp^2 carbons), 167.4 (C=O), 183.1 (C=O) ppm; MS (EI) m/z 177 (M^+ + 2, characteristic peak for *o*-quinone compounds[14]), 175 (M^+). Anal. Calcd for $C_{10}H_9NO_2$: C, 68.56; H, 5.18; N, 8.00. Found: C, 68.19; H, 5.03; N, 7.89.

2-Carboxy-3,7-dimethyl-5-benzyloxyindole (21). The starting material **20** was prepared from 2-methyl-4-benzyloxyaniline and methyl α-ethylacetoacetate according to the established procedure (combination of a Jaap−Klingemann reaction and a Fischer indolization).[12,13] 2-Methyl-4-benzyloxyaniline was prepared from 2-methyl-4-hydroxyaniline by the standard procedures of amino acetylation with acetic anhydride, benzylation of the hydroxy group with benzyl chloride, and deprotection of the acetamide group with NaOH(aq).[21] The indole derivative **20** (104 mg, 0.336 mmol) was treated in 1 *N* KOH aqueous CH_3OH solution (11 mL, 1:1, v/v) at 80 °C for 1 h. Acidification of the reaction mixture to pH 3 with 1 *N* HCl gave a white precipitate that was isolated by centrifugation and dried *in vacuo* (100%): mp 208−211 °C; IR (KBr) 3484 (NH), 2924 (−COOH), 1660 cm^{-1} (C=O); ^1H NMR ($CDCl_3$) δ 2.2 (1 H, br), 2.48 (3 H, s), 2.61 (3 H, s), 5.11 (2 H, s), 6.95 (1 H, s), 6.97 (1 H, s), 7.33−7.50 (5 H, m), 8.56 (1 H, br s); MS (EI) m/z 295 (M^+), 204 (M^+-CH_2Ph).[20]

(21) Greene, T. W. *Protective Groups in Organic Synthesis*; John Wiley & Sons: New York, 1981.

3,7-Dimethyl-5-(benzyloxy)indole (22). A mixture of **21** (280 mg, 0.95 mmol) and $2CuO-Cr_2O_3$ (35.5 mg, 0.114) in quinoline (5.6 mL) was heated at 200 °C for 2 h. The mixture was then extracted with ether and washed with 1 *N* HCl and saturated $NaHCO_3$(aq). The organic layer was further washed with 1 *N* HCl several times and dried over $MgSO_4$. After removal of $MgSO_4$ by filtration, evaporation of the solvent gave a crude product from which **22** was isolated by flash column chromatography (SiO_2, *n*-hexane−AcOEt = 20:1) in a 94% yield: mp 102−104 °C; IR (KBr) 3412 cm^{-1} (NH); ^1H NMR ($CDCl_3$) δ 2.29 (3 H, s), 2.44 (3 H, s), 5.11 (2 H, s), 6.78 (1 H, s), 6.96 (2 H, s), 7.31−7.50 (5 H, m), 7.72 (1 H, br s); MS (EI) *m*/*z* 251 (M^+), 160 (M^+-CH_2Ph). Anal. Calcd for $C_{17}H_{17}NO$: C, 81.24; H, 6.82; N, 5.57. Found: C, 81.03; H, 6.95; N, 5.47.

3,7-Dimethyl-5-hydroxyindole (23). The benzyl group of **22** (170 mg, 0.676 mmol) was removed by catalytic hydrogenation using 10% Pd−C (47.5 mg) under H_2 (4 atm) at 60 °C in CH_3OH (59 mL) for 11 h. After removal of the catalyst by filtration, evaporation of the solvent gave a product quantitatively: mp 182−183 °C; IR (KBr) 3456 and 3396 (NH and OH); ^1H NMR ($CDCl_3$) δ 2.26 (3 H, s), 2.43 (3 H, s), 4.48 (1 H, br s), 6.60 (1 H, s), 6.82 (1 H, d, J = 1.6 Hz), 6.96 (1 H, s), 7.78 (1 H, br s); MS (EI) *m*/*z* 161 (M^+).[20]

3,7-Dimethylindole-4,5-dione (5). To a CH_3CN solution (44 mL) of **23** (109 mg, 0.676 mmol) was added Fremy's salt (736 mg, 2.71 mmol) in 0.062 *M* KH_2PO_4 aqueous solution (44 mL) at 0 °C. The mixture was stirred at 0 °C for 15 min and at room temperature for 2 h. The final reaction mixture was then extracted with $CHCl_3$. After the extract was dried over $MgSO_4$, evaporation of the solvent gave a dark brown residue from which quinone **5** was isolated in a 76% yield by flash column chromatography (SiO_2, $CHCl_3$): mp >300 °C; IR (KBr) 3128 (NH), 1644 cm^{-1} (C=O); ^1H NMR (DMSO-d_6) δ 2.18 (6 H, s), 5.78 (1 H, s), 6.82 (1 H, s), 11.89 (1 H, br s); ^{13}C NMR (DMSO-d_6) 11.1, 17.3, 118.9, 121.5, 121.7, 123.3, 137.3, 145.8 (6 sp^2 carbons), 174.8 (C=O), 183.3 (C=O) ppm; MS (EI) *m*/*z* 177 ($M^+ + 2$, characteristic peak for *o*-quinone compounds[14]), 175 (M^+). Anal. Calcd for $C_{10}H_9NO_2$: C, 68.56; H, 5.18; N, 8.00. Found: C, 68.30; H, 5.10; N, 7.85 (a sample for the elemental analysis was obtained by recrystallization from CH_3CN).

Kinetic Analysis. The reactions of the indolequinone and several amines were followed by the UV−vis spectra under the pseudo-first-order conditions with excess amine in deaerated CH_3OH at 30 °C. Typically, a CH_3OH solution of the indolequinone (5.0×10^{-5} M) was placed in a UV cell (1 cm path length, sealed tightly with a silicon rubber cap) and deaerated by bubbling Ar through it for ca. 20 min. Then the amine was added with a microsyringe to start the reaction. The pseudo-first-order rate constant (k_{obs}) was calculated from the rate of a decrease in the intensity of the absorption band due to the quinone or an increase in the intensity of the absorption band due to the products. The nonlinear curve-fitting program (Mac curve fit) was used to determine the rate constants in case the final value of the absorbance (A_∞) was obscured by the followup reaction.

Catalytic Oxidation of Benzylamine under O_2. The catalytic oxidation of benzylamine with molecular oxygen was initiated by adding the amine (1.0 mmol) with a microsyringe to an O_2-saturated CH_3OH solution (10 mL) of the indolequinone (1 mM), when the initial amine concentration was 100 mM. The mixture was stirred at room temperature under O_2 atmosphere. The rate of formation of benzaldehyde was monitored by HPLC [pump: Hitachi L-600; UV detector: Hitachi 638-0430 UV monitor; column: Waters radial compression separation system (C_{18}); eluent: $CH_3OH/H_2O/H_3PO_4$, 45:54.5:0.5]. The ^1H NMR and IR spectra of the concentrated final reaction mixture indicated that *N*-benzylidenebenzylamine ($PhCH_2N=CHPh$) was formed quantitatively: ^1H NMR ($CDCl_3$) δ 4.80 (2 H, s, $-CH_2-$), 7.20−7.50 (8 H, m, aromatic protons), 7.70−7.85 (2 H, m, aromatic protons), 8.38 (1 H, br s, −CH=); IR (neat) 1648 cm^{-1} (C=N).

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Supporting Information Available: Copies of the ^1H-NMR spectra of the synthetic intermediates **8**, **10**, **11**, **13**, **15**, **17**, **19**, **21**, and **23** (9 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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