A Model Compound of the Novel Organic Cofactor CTQ (Cysteine Tryptophylquinone) of Quinohemoprotein Amine Dehydrogenase

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Keywords: Amine dehydrogenases / Cofactors / Post-translational modification / Quinones / Quinoprotein

A model compound of the novel organic cofactor CTQ (cysteine tryptophylquinone) of quinohemoprotein amine dehydrogenase (QH-AmDH) has been synthesized for the first time by the Michael addition of phenylmethanethiol to the 3-methyl-6,7-indolequinone derivative as a key step. The model compound replicates the characteristic absorption spectrum of native CTQ as well as its redox potential in the γ subunit of QH-AmDH. Detailed structural and spectroscopic characterizations of the model compound have provided important insights into the substituent effects of the thioether group of CTQ.

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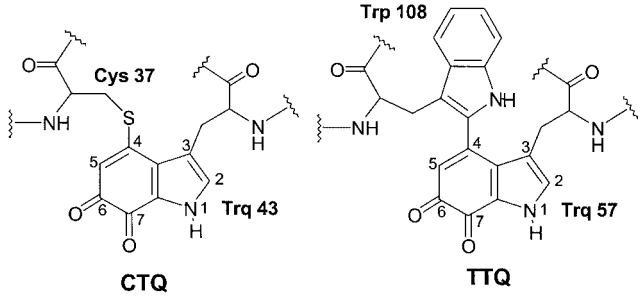
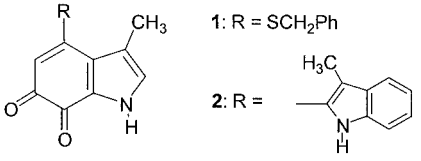
Introduction

Quinohemoprotein amine dehydrogenase (QH-AmDH) is a bacterial enzyme, isolated from *Pseudomonas putida* and *Paracoccus denitrificants*, that catalyses the oxidative deamination of primary amines to the corresponding aldehydes and transfers the substrate-derived electrons to physiological electron acceptors such as cytochrome *c*-550 and azurin.[1,2] The enzyme is an αβγ heterotrimer, in which there are two heme *c* prosthetic groups in the largest α subunit and a carbonyl cofactor in the smallest γ subunit.[1,2] Two independent research groups have recently analysed the enzyme by X-ray crystallography and identified the organic cofactor as a tryptophan-6,7-quinone (Trq 43) covalently attached to the thiol group of cysteine (Cys 37) at its C-4 position, and is thus called CTQ (cysteine tryptophylquinone).[3,4]



CTQ is a new member of the redox-active amino acid cofactors that include TPQ (topa quinone) of the copper-containing amine oxidases,[5] LTQ (lysine tyrosylquinone) of lysyl oxidase,[6] Tyr-Cys (tyrosine covalently attached to the thiol group of cysteine) of galactose oxidase,[7] and TTQ (tryptophan tryptophylquinone) of methylamine and aromatic amine dehydrogenases.[8] Among such post-translationally derived organic cofactors, CTQ is closely related to TTQ, since both cofactors possess the same indole-6,7-quinone structure. Therefore, the oxidative deamination reaction of QH-AmDH is considered to occur at the quinone moiety of CTQ, as is the case for the TTQ-dependent amine dehydrogenases.[9] However, detailed spectroscopic characterization of CTQ as well as kinetic studies of the enzymatic reaction have been hampered by the strong absorption bands arising from the heme prosthetic groups in the α subunit. Recently, Kano and co-workers succeeded in isolating the CTQ-containing γ subunit and characterized the native CTQ cofactor by spectroscopic and electrochemical techniques.[10] Nevertheless, a limited quantity of the sample as well as the complexity of its structure due to the peptide backbone have prevented a more detailed examination of the chemistry of the CTQ cofactor.

In this paper, we report the synthesis and characterization of the first model compound of CTQ having exactly the same indole-6,7-quinone skeleton with a thioether group at the C-4 position. Comparison of the spectroscopic and redox properties of the CTQ model compound **1** and the TTQ model compound **2**[11,12] has provided important insights into the electronic effects of C-4 substituents of indole-6,7-quinone cofactors.



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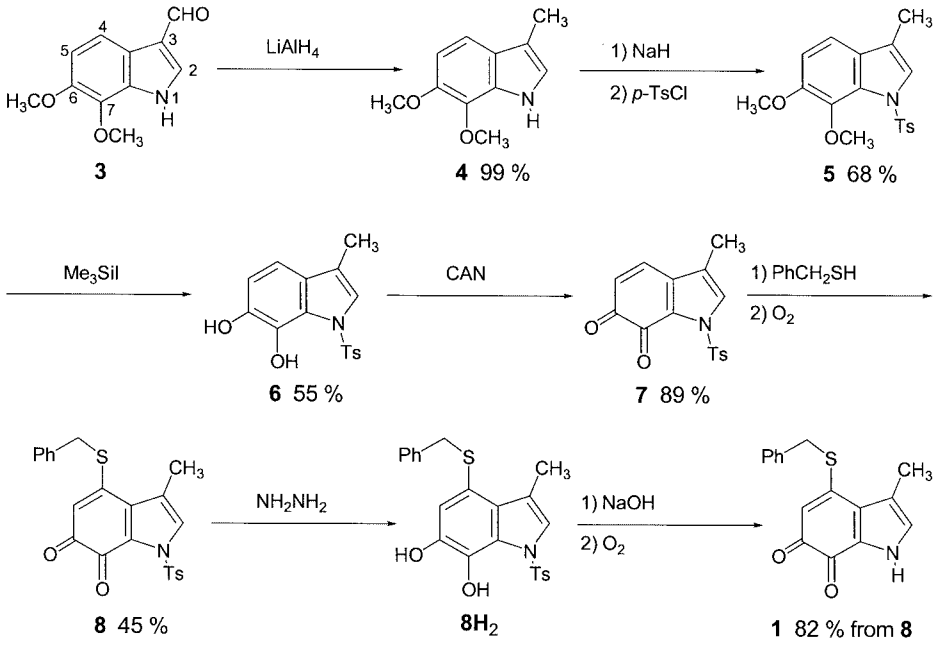
Results and Discussion

The CTQ model compound **1** has been synthesized as outlined in Scheme 1. Reduction of the formyl group of known compound **3**[13,14] by $LiAlH_4$ gave compound **4**. The indole nitrogen atom (N-1) of **4** was then protected by a *p*-tosyl group to give compound **5**. Deprotection of the methoxy groups of **5** by trimethylsilyl iodide gave quinol **6**,[15] which was converted into indole-6,7-quinone derivative **7** by oxidation with ceric ammonium nitrate (CAN). Treatment of **7** with 1 equiv. of phenylmethanethiol in DMF produced the Michael adduct of the mercaptan (**$8H_2$**: the quinol derivative of **8**) together with quinol **6**.[16] The quinol mixture was subjected to column chromatography on SiO_2 which induced air oxidation of the quinols to provide quin-



Scheme 1

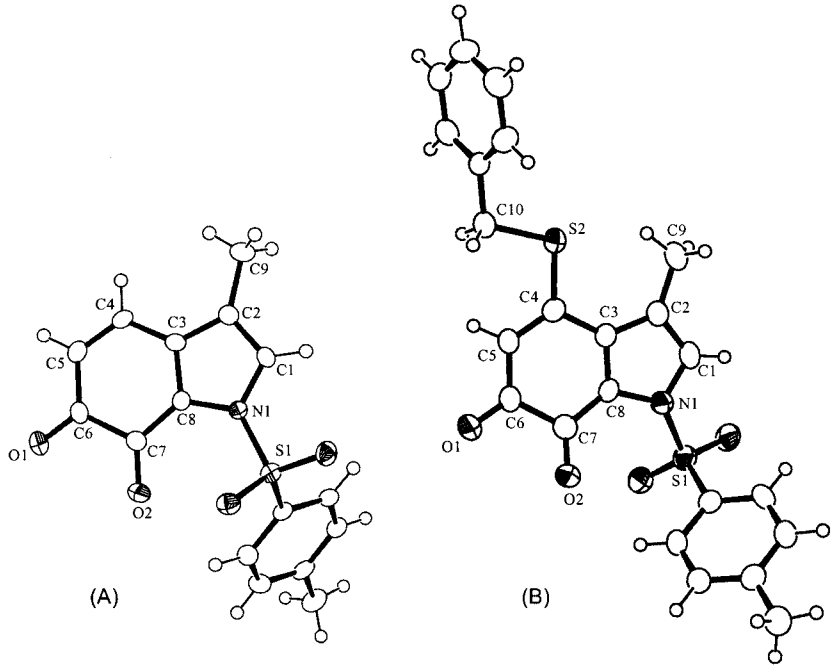


Figure 1. ORTEP drawings of compound **7** (A) and compound **8** (B) showing 50% probability thermal ellipsoids

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one **8** together with the recovery of some **7**. Deprotection of the tosyl group had to be carried out on quinol $\mathbf{8H_2}$, since quinone **8** was not stable enough under the basic conditions (NaOH in ethanol) of the deprotection reaction. Thus, compound **8** was first converted into $\mathbf{8H_2}$ by reduction with NH_2NH_2, and then the resulting quinol $\mathbf{8H_2}$ was treated with NaOH in ethanol to give the deprotected product. Aerobic work up of the deprotected product gave the CTQ model compound **1** as a dark red solid.[17]

The crystal structures of the *o*-quinone derivatives **7** and **8** (synthetic intermediates) have been determined by X-ray crystallographic analysis, as shown in Figure 1. Selected bond lengths and angles are summarized in Table 1. Although the indole nitrogen atoms (N-1) of these compounds are protected by the tosyl group, comparison of the detailed structures of **7** and **8** has provided an insight into the substituent effects of the thioether group. Namely, the deviation from planarity of the quinone moiety O(1)−C(6)−C(7)−O(2) of **8** (dihedral angle = 14.4°) is larger than that of **7** (9.6°), while the C(10)−S(2)−C(4)−C(5)−C(6)−O(1) framework of **8** is nearly flat [the dihedral angles of C(10)−S(2)−C(4)−C(5), S(2)−C(4)−C(5)−C(6), and C(4)−C(5)−C(6)−O(1) are 2.5°, 3.6°, and 2.1°, respectively]. The C(5)−C(6) bond of **8** (1.437 Å) is shorter than that of **7** (1.468 Å), while the C(4)−C(5) and C(6)−O(1) bonds of **8** (1.357 and 1.221 Å, respectively) are longer than those of **7** (1.337 and 1.215 Å, respectively). Moreover, the S(2)−C(4) bond of **8** (1.720 Å) is slightly shorter than the typical S−C bond in simple thioanisole derivatives (1.77 Å).[18,19] These structural features clearly suggest that there is a conjugative interaction between S(2) and the α,β-unsaturated carbonyl moiety [C(4)=C(5)−C(6)=O(1)] of compound **8**. This conclusion is supported by the IR data; the C=O stretching vibration of quinone **8** appears at 1636 cm^{-1}, which is lower than that of compound **7** by about 30 cm^{-1} (see Exp. Sect.).

Figure 2 shows the UV/Vis spectrum of compound **1** (solid line) which exhibits two distinct π−π* transition bands at 343 and 381 nm (ε = 14800 and 12000 $M^{-1}\cdot cm^{-1}$, respectively) together with a weak n−π* transition band at 450−600 nm. These absorption bands are very similar to those of native CTQ [350−380 nm (π−π*) and 450−600 nm (n−π*)], although the absorption band at ca. 350 nm of native CTQ is not well separated from the band at 380 nm.[10]

The absorption bands in the visible region (λ > 350 nm) of **1** are blue-shifted relative to those of the TTQ model compound **2** (π−π*: 407 nm, ε = 10700 $M^{-1}\cdot cm^{-1}$; n−π*: 500−650 nm), and the C=O stretching vibration in the IR spectrum of quinone **1** (1630 cm^{-1}) is slightly higher than that of quinone **2** (1628 cm^{-1}).[11,12] These differences in $\lambda_{max.}$ and $\nu_{C=O}$ may reflect the different levels of conjugative interaction between the quinone ring and the C-4 substituent (thioether group in **1** vs. indole ring in **2**) of **1** and **2**. In addition, the lower value of $\nu_{C=O}$ (1630 cm^{-1}) of **1** compared with that of **8** (1636 cm^{-1}) suggests that there is also a conjugative interaction between N-1 and the C-7 carbonyl group of compound **1**.

Table 1. Selected bond lengths [Å] and angles [°] of compounds **7** and **8**

Compound **7**			
C(1)−C(2)	1.373(4)	C(2)−C(3)	1.416(4)
C(2)−C(9)	1.504(4)	C(3)−C(4)	1.457(4)
C(3)−C(8)	1.391(3)	C(4)−C(5)	1.337(4)
C(5)−C(6)	1.468(4)	C(6)−C(7)	1.560(4)
C(7)−C(8)	1.440(4)	S(1)−N(1)	1.709(2)
O(1)−C(6)	1.215(3)	O(2)−C(7)	1.219(3)
N(1)−C(1)	1.379(3)	N(1)−C(8)	1.398(3)
C(1)−C(2)−C(3)	106.2(2)	C(1)−C(2)−C(9)	126.1(3)
C(3)−C(2)−C(9)	127.7(3)	C(2)−C(3)−C(4)	129.9(2)
C(2)−C(3)−C(8)	108.7(2)	C(4)−C(3)−C(8)	121.4(3)
C(3)−C(4)−C(5)	121.2(2)	C(4)−C(5)−C(6)	120.7(3)
O(1)−C(6)−C(5)	122.9(3)	O(1)−C(6)−C(7)	117.5(3)
C(5)−C(6)−C(7)	119.6(3)	O(2)−C(7)−C(6)	118.5(3)
O(2)−C(7)−C(8)	127.4(3)	C(6)−C(7)−C(8)	114.0(2)
N(1)−C(8)−C(3)	107.1(2)	N(1)−C(8)−C(7)	130.2(2)
C(3)−C(8)−C(7)	122.7(3)	S(1)−N(1)−C(1)	122.3(2)
S(1)−N(1)−C(8)	129.4(2)	C(1)−N(1)−C(8)	107.9(2)
N(1)−C(1)−C(2)	110.1(3)		
Compound **8**			
C(1)−C(2)	1.351(7)	C(2)−C(3)	1.416(7)
C(2)−C(9)	1.497(7)	C(3)−C(4)	1.476(7)
C(3)−C(8)	1.387(7)	C(4)−C(5)	1.357(7)
C(5)−C(6)	1.437(7)	C(6)−C(7)	1.550(7)
C(7)−C(8)	1.438(7)	O(1)−C(6)	1.221(6)
O(2)−C(7)	1.207(6)	S(1)−N(1)	1.707(4)
N(1)−C(1)	1.380(7)	N(1)−C(8)	1.403(6)
S(2)−C(4)	1.720(5)	S(2)−C(10)	1.815(5)
C(1)−C(2)−C(3)	106.7(5)	C(1)−C(2)−C(9)	123.8(5)
C(3)−C(2)−C(9)	129.5(5)	C(2)−C(3)−C(4)	132.2(5)
C(2)−C(3)−C(8)	108.0(4)	C(4)−C(3)−C(8)	119.8(4)
C(3)−C(4)−C(5)	119.7(4)	S(2)−C(4)−C(3)	116.2(4)
C(5)−C(4)−S(2)	124.1(4)	C(4)−C(5)−C(6)	122.5(5)
O(1)−C(6)−C(5)	123.5(5)	O(1)−C(6)−C(7)	117.4(5)
C(5)−C(6)−C(7)	119.0(4)	O(2)−C(7)−C(6)	119.1(4)
O(2)−C(7)−C(8)	127.0(5)	C(6)−C(7)−C(8)	113.8(4)
N(1)−C(8)−C(3)	107.5(4)	N(1)−C(8)−C(7)	128.2(4)
C(3)−C(8)−C(7)	123.9(4)	S(1)−N(1)−C(1)	121.8(3)
S(1)−N(1)−C(8)	128.3(3)	C(1)−N(1)−C(8)	106.8(4)
N(1)−C(1)−C(2)	110.8(4)	C(4)−S(2)−C(10)	103.2(2)

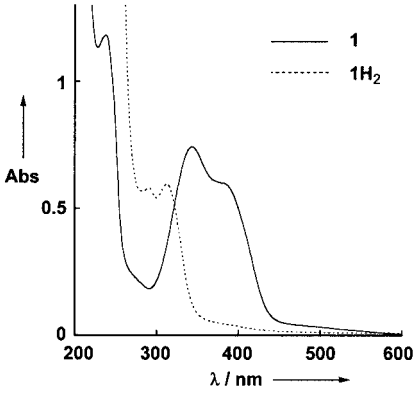

Figure 2. UV/Vis spectra of **1** (5.0×10^{-5} M) and its quinol derivative $\mathbf{1H_2}$, generated by the reduction of **1** with $MeNHNH_2$ (10 equiv.) in CH_3CN

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Compound **1** exhibits a quasi-reversible redox couple at $E°_{1/2} = -0.187$ V vs. Ag/AgCl in an aqueous solution containing 30% CH_3CN (pH = 7.0), which is similar to that of native CTQ (−0.132 V vs. Ag/AgCl at pH = 7.0)[10] but somewhat negative compared with that of the TTQ model compound **2** (−0.090 V vs. Ag/AgCl at pH = 7.0).[12] The negative shift of $E°_{1/2}$ reflects the electron-donating nature of the thioether group of compound **1**.

Reduction of **1** to its quinol form $\mathbf{1H_2}$ with methylhydrazine caused a decrease in the visible absorption bands together with an increase of the absorption bands at around 300 nm [$\lambda_{max.}$ = 291 nm (ε = 11600 $M^{-1}\cdot cm^{-1}$) and 312 (11900)] (dotted line in Figure 2). The spectral change due to the two-electron reduction of **1** to $\mathbf{1H_2}$ is also very similar to that observed for native CTQ.[10]

Conclusion

We have succeeded in synthesizing the first model compound of the novel organic cofactor CTQ of QH-AmDH, which has exactly the same indole-6,7-quinone skeleton with a thioether group at the C-4 position. The compound replicates the characteristic absorption spectrum of CTQ as well as its redox potential of the γ subunit. The model compound has been synthesized by the nucleophilic attack (Michael addition) of a thiol at the C-4 position of indole-6,7-quinone **7**. This kind of reaction may be involved in the biogenetic pathway of CTQ in the QH-AmDH enzymatic system.[20] Amine oxidation by the model compound is currently being investigated in order to shed light on the catalytic mechanism of QH-AmDH.

Experimental Section

General: The reagents and solvents used in this study were commercial products of the highest available purity and were further purified by standard methods, if necessary.[21] Melting points were determined with a Yanaco Micro Melting Point Apparatus. FT-IR spectra were recorded with a Shimadzu FTIR-8200PC and UV/Vis spectra were taken with a Hewlett−Packard 8453 photodiode array spectrophotometer. ^{1}H NMR spectra were recorded with a JEOL FT NMR Lambda 300WB or a JEOL FT NMR GX-400 spectrometer. Mass spectra were recorded with a JEOL JMS-700T Tandem MS-station mass spectrometer. Elemental analyses were performed with a Fisons Instrument EA1108 Elemental Analyzer. The cyclic voltammetry measurement was performed with an ALS 630 electrochemical analyzer in a deaerated 0.10 M phosphate buffer solution containing 30% CH_3CN. The working electrode was a glassy carbon electrode (BAS) which was polished with BAS polishing alumina suspension and rinsed with H_2O and dried before use. The counter electrode was a platinum wire and the reference electrode was Ag/AgCl.

X-ray Structure Determination: The single crystals of compounds **7** and **8** were mounted on a glass fiber. X-ray diffraction data were collected with a Rigaku RAXIS-RAPID imaging plate two-dimensional area detector using graphite-monochromated Mo-K_α radiation (λ = 0.71069 Å) and $2\theta_{max.}$ = 55.0°. All the crystallographic calculations were performed by using the Crystal Structure software package.[22] The crystal structures were solved by direct methods and refined by the full-matrix least-squares method using SIR-92. All non-hydrogen atoms and hydrogen atoms were refined anisotropically and isotropically, respectively. X-ray crystallographic data are presented in Table 2. Crystallographic data for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-232604 (**7**) and -232605 (**8**). Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [Fax: (internat.) + 44-1223/336-033; E-mail: deposit@ccdc.cam.ac.uk].

Table 2. Summary of the X-ray crystallographic data for compounds **7** and **8**

	7	**8**
Empirical formula	$C_{16}H_{13}NO_4S$	$C_{23}H_{19}O_4NS_2$
Formula mass	315.34	437.53
Crystal system	monoclinic	orthorhombic
Space group	$P2_1/c$ (No. 14)	$Pbca$ (No. 61)
a [Å]	6.970(1)	7.356(2)
b [Å]	8.451(1)	17.053(6)
c [Å]	24.229(3)	31.49(1)
β [°]	95.464(5)	90
V [Å^3]	1420.6(4)	3950(2)
Z	4	8
F(000)	656.00	1824.00
$D_{calcd.}$ [g·cm^{-3}]	1.474	1.471
T [K]	159	164
Crystal size [mm]	0.12 × 0.13 × 0.10	0.10 × 0.17 × 0.08
μ (Mo-K_α) [cm^{-1}]	2.46	3.02
$2\theta_{max.}$ [°]	55.0	55.0
No. of reflns. measd.	13275	50266
No. of reflns. obsd.	2133 [$I > 1.00\sigma(I)$]	3124 [$I > 0.50\sigma(I)$]
No. of variables	213	291
R[a]	0.035	0.050
Rw[b]	0.050	0.088
GOF	1.00	1.00

[a] $R = \Sigma||F_o| - |F_c||/\Sigma|F_o|$. [b] $R_w = [\Sigma w(|F_o| - |F_c|)^2/\Sigma wF_o^2]^{1/2}$.

6,7-Dimethoxy-3-methylindole (4): 6,7-Dimethoxyindole-3-carbaldehyde (**3**) was prepared from vanillin in 7 steps according to reported procedures.[13,14] Compound **3** (465 mg, 2.3 mmol) in dry THF (23 mL) was added dropwise to $LiAlH_4$ (175 mg, 4.6 mmol), suspended in dry THF (12 mL) at 0 °C under N_2, and the mixture was stirred at reflux temperature for 6 h. The reaction was quenched by adding saturated aq. Na_2SO_4, and insoluble materials were removed by filtration. The filtrate was then extracted with diethyl ether (30 mL × 4), and the combined organic layers were dried with $MgSO_4$. After removal of $MgSO_4$ by filtration, evaporation of the solvent gave a crude product, from which **4** was isolated as a yellow-brown oily material in 99% yield by column chromatography (SiO_2; $CHCl_3$). IR (neat): $\tilde{\nu}$ = 3415 (NH), 1301 and 1207 (C−O) cm^{-1}. ^{1}H NMR ($CDCl_3$): δ = 2.29 (s, 3 H, CH_3), 3.92 (s, 3 H, OCH_3), 3.98 (s, 3 H, OCH_3), 6.85 (d, J = 8.6 Hz, 1 H, 5-H), 6.88 (br. s, 1 H, 2-H), 7.21 (d, J = 8.6 Hz, 1 H, 4-H), 7.97 (br. s, 1 H, NH) ppm. ^{13}C NMR ($CDCl_3$): δ = 9.7 (CH_3), 57.5, 60.8 (OCH_3), 107.9, 111.9, 113.7, 121.2, 125.2, 130.7, 134.4, 147.0 (8 aromatic C) ppm. HRMS (FAB): m/z = 191.0948 [M$^+$]; calcd. for

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$C_{11}H_{13}NO_2$ 191.0947. $C_{11}H_{13}NO_2$ (191.2): calcd. C 69.09, H 6.85, N 7.32; found C 69.07, H 6.96, N 7.17.

6,7-Dimethoxy-3-methyl-1-(*p*-tosyl)indole (5): Compound **4** (1.00 g, 5.2 mmol) in dry DMF (4.0 mL) was added dropwise to NaH (318 mg, 11 mmol, 65% content in a mineral oil suspension that was washed with dry *n*-hexane three times before use) suspended in dry DMF (10 mL) at room temperature under N_2, and the mixture was stirred for 10 min. A dry DMF (3.0 mL) solution of *p*-toluenesulfonyl chloride (1.95 g, 11 mmol) was then added to the mixture, and the resulting solution was stirred at 0 °C for 2 h. The reaction was quenched by adding 20% aq. Na_2CO_3 (100 mL), and the mixture was extracted with EtOAc (30 mL × 5). After drying with $MgSO_4$, removal of the solvent gave a crude product, from which compound **5** was isolated as a pale green solid in 68% yield by column chromatography (SiO_2; *n*-hexane/EtOAc, 9:1). M.p. 77−79 °C. IR (neat): $\tilde{\nu}$ = 1354 and 1173 (SO_2), 1258 and 1038 (C−O) cm^{-1}. ^1H NMR ($CDCl_3$): δ = 2.23 (d, *J* = 1.3 Hz, 3 H, 3-CH_3), 2.35 (s, 3 H, $C_6H_4CH_3$), 3.83 (s, 3 H, OCH_3), 3.86 (s, 3 H, OCH_3), 6.89 (d, *J* = 8.5 Hz, 1 H, 5-H), 7.11 (d, *J* = 8.5 Hz, 2 H, 4-H), 7.22 (d, *J* = 8.3 Hz, 2 H, $C_6H_4CH_3$), 7.48 (d, *J* = 1.3 Hz, 1 H, 2-H), 7.76 (d, *J* = 8.3 Hz, 2 H, $C_6H_4CH_3$) ppm. ^{13}C NMR ($CDCl_3$): δ = 9.5 (CH_3), 21.5 ($C_6H_4CH_3$), 56.7 (OCH_3), 60.6 (OCH_3), 109.6, 113.9, 116.0, 121.1, 125.0, 127.3, 128.7, 129.5, 136.7, 137.0, 143.9, 150.6 (12 aromatic C) ppm. HRMS (FAB): *m*/*z* = 345.1049 [M^+]; calcd. for $C_{18}H_{19}NO_4S$ 345.1036. $C_{18}H_{19}NO_4S$ (345.4): calcd. C 62.59, H 5.54, N 4.06; found C 62.35, H 5.60, N 4.26.

3-Methyl-1-(*p*-tosyl)indole-6,7-diol (6): Compound **5** (253 mg, 0.73 mmol) was treated with trimethylsilyl iodide (2.08 mL, 15 mmol) in dry CH_3CN (10 mL) at reflux temperature under N_2 for 30 h. The reaction was quenched by adding water (30 mL) and the mixture was extracted with EtOAc (30 mL × 5). The combined organic layers were washed with aqueous $Na_2S_2O_3$ and dried with $MgSO_4$. After removal of the $MgSO_4$ by filtration, evaporation of the solvent gave a crude material, from which compound **6** was isolated in 55% yield by column chromatography (SiO_2; *n*-hexane/EtOAc, 7:1). M.p. 162−163 °C. IR (KBr): $\tilde{\nu}$ = 3449 (OH), 1261 and 1088 (SO_2) cm^{-1}. ^1H NMR ($CDCl_3$): δ = 2.13 (d, *J* = 1.2 Hz, 3 H, 3-CH_3), 2.35 (s, 3 H, $C_6H_5CH_3$), 5.71 (s, 1 H, OH), 6.83 (d, *J* = 8.3 Hz, 1 H, 5-H), 6.94 (d, *J* = 8.3 Hz, 1 H, 4-H), 7.03 (d, *J* = 1.2 Hz, 1 H, 2-H), 7.22 (d, *J* = 8.1 Hz, 2 H, $C_6H_4CH_3$), 7.63 (d, *J* = 8.1 Hz, 2 H, $C_6H_4CH_3$), 8.96 (s, 1 H, OH) ppm. HRMS (FAB): *m*/*z* = 317.0723 [M^+]; calcd. for $C_{16}H_{15}NO_4S$ 317.0723.

3-Methyl-1-(*p*-tosyl)indole-6,7-dione (7): CAN (86.3 mg, 0.16 mmol) was added to an acetonitrile solution (4.0 mL) of **6** (50.1 mg, 0.16 mmol), containing 1.0 mL of water at 0 °C. The mixture was stirred at 0 °C for 10 min. The reaction was then quenched by adding aqueous K_2CO_3, and the mixture was extracted with CH_2Cl_2 (20 mL × 4) and dried with $MgSO_4$. After removal of the $MgSO_4$ by filtration, evaporation of the solvent gave a red solid, from which compound **7** was isolated in 89% yield by column chromatography (SiO_2; $CHCl_3$). M.p. 197−198 °C. IR (KBr): $\tilde{\nu}$ = 1663 (C=O), 1377 and 1092 (SO_2) cm^{-1}. ^1H NMR ($CDCl_3$): δ = 2.16 (s, 3 H, 3-CH_3), 2.42 (s, 3 H, $C_6H_5CH_3$), 6.11 (d, *J* = 10.0 Hz, 1 H, 5-H), 7.22 (d, *J* = 10.0 Hz, 1 H, 4-H), 7.33 (d, *J* = 8.6 Hz, 2 H, $C_6H_4CH_3$), 7.60 (s, 1 H, 2-H), 8.08 (d, *J* = 8.6 Hz, 2 H, $C_6H_4CH_3$) ppm. ^{13}C NMR ($CDCl_3$): δ = 8.67 (CH_3), 21.14 ($C_6H_4CH_3$), 120.6, 125.9, 126.2, 128.4, 129.9, 130.9, 133.4, 134.9, 135.4, 146.1 (10 aromatic C), 165.3, 181.0 (C=O) ppm. HRMS (FAB): *m*/*z* = 316.0649 [M + H]$^+$; calcd. for $C_{16}H_{14}NO_4S$ 316.0644. $C_{16}H_{13}NO_4S$ (315.3): calcd. C 60.94, H 4.16, N 4.44; found C 61.15, H 3.96, N 4.49.

4-(Benzylsulfanyl)-3-methyl-1-(*p*-tosyl)indole-6,7-dione (8): Phenylmethanethiol (7.44 μL, 0.063 mmol) was added to a dry DMF solution (4.0 mL) of **7** (20.0 mg, 0.063 mmol) at room temperature under N_2, and the resulting mixture was stirred for 2.5 h. The reaction mixture was then diluted with water (50 mL), extracted with EtOAc (20 mL × 3) and dried with $MgSO_4$. After removal of the $MgSO_4$ by filtration, evaporation of the solvent gave a reddish brown solid, from which compound **8** was isolated as a red solid in 45% yield by column chromatography (SiO_2; $CHCl_3$) under air. M.p. 242−244 °C (dec.). IR (KBr): $\tilde{\nu}$ = 1636 (C=O), 1385 and 1180 (SO_2) cm^{-1}. ^1H NMR ($CDCl_3$): δ = 2.34 (s, 3 H, 3-CH_3), 2.42 (s, 3 H, $C_6H_4CH_3$), 4.17 (s, 2 H, $SCH_2C_6H_5$), 6.05 (s, 1 H, 5-H), 7.33−7.39 (m, 7 H, $C_6H_4CH_3$, $SCH_2C_6H_5$), 7.63 (s, 1 H, 2-H), 8.05 (d, *J* = 8.6 Hz, 2 H, $C_6H_4CH_3$) ppm. HRMS (FAB): *m*/*z* = 439.0917 [M + 2 H]$^+$; calcd. for $C_{23}H_{21}NO_4S_2$ 439.0913. $C_{23}H_{19}NO_4S_2$ + 1/6H_2O: calcd. C 62.71, H 4.42, N 3.18; found C 62.63, H 4.37, N 3.08.

4-(Benzylsulfanyl)-3-methylindole-6,7-dione (1): Hydrazine monohydrate (5.5 μL, 0.11 mmol, 5 equiv.) was added to a suspension of **8** (10.0 mg, 0.023 mmol) in dry CH_3CN (3 mL, deoxygenated by bubbling N_2 for 10 min), and the reaction mixture was stirred under anaerobic conditions (N_2) for 20 min. Removal of the solvent under reduced pressure gave a yellow solid material (**$8H_2$**), to which 3 N NaOH in H_2O/EtOH (9:5, v/v) (278 μL) was added. The mixture was then stirred at room temperature for 30 min, and was extracted with EtOAc (15 mL × 6). After the extract was dried with $MgSO_4$, evaporation of the solvent gave a dark red residue, from which compound **1** was isolated in 82% yield by column chromatography (SiO_2; $CHCl_3$/EtOAc, 3:2). M.p. 239−240 °C. IR (KBr): $\tilde{\nu}$ = ca. 3500 (br. N−H), 1630 (C=O), 700 (S−C) cm^{-1}. ^1H NMR ([D_6]DMSO): δ = 2.19 (s, 3 H, 3-CH_3), 4.39 (s, 2 H, $SCH_2C_6H_5$), 5.94 (s, 1 H, 5-H), 7.13 (s, 1 H, 2-H), 7.29−7.48 (m, 5 H, C_6H_5), 12.7 (br. s, 1 H, NH) ppm. ^{13}C NMR ([D_6]DMSO): δ = 13.0 (CH_3), 35.4 (SCH_2Ph), 114.1, 119.7, 125.0, 127.7, 127.9, 128.7, 129.2, 129.7, 134.8, 156.8 (10 aromatic C), 167.9, 178.7 (C=O) ppm. HRMS (FAB): *m*/*z* = 284.0743 [M + H]$^+$; calcd. for $C_{16}H_{14}NO_2S$ 284.0746. $C_{16}H_{13}NO_2S$ + 1/4$CHCl_3$: calcd. C 62.32, H 4.26, N 4.47; found C 62.54, H 4.46, N 4.33.

4-(Benzylsulfanyl)-3-methylindole-6,7-diol (1H_2): Methylhydrazine (3.4 μL, 6.4 × 10^{-2} mmol), was added to a deaerated CH_3CN solution (2.0 mL) of **1** (1.81 mg, 6.39 × 10^{-3} mmol) and the reaction mixture was stirred under anaerobic conditions (N_2) for 30 min. Removal of the solvent under reduced pressure gave a dark yellow material of **1H_2** (1.5 mg, 83% yield). ^1H NMR ([D_6]DMSO): δ = 2.20 (s, 3 H, 3-CH_3), 3.97 (s, 2 H, SCH_2Ph), 6.59 (s, 1 H, 5-H), 6.82 (s, 1 H, 2-H), 7.18−7.28 (m, 5 H, $SCH_2C_6H_5$), 10.3 (s, 1 H, NH) ppm. MS (EI): *m*/*z* = 285 [M^+].

Acknowledgments

This work was supported in part by Grants-in-Aid for Scientific Research from the Ministry of Education, Science, Sports, and Culture of Japan (13480189 and 15350105). The authors also acknowledge Professor Kenji Kano of Kyoto University and Professor Ken Hirotsu of Osaka City University for their helpful discussions.

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Received March 23, 2004

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