Structures of Cordypyridones A−D, Antimalarial *N*-Hydroxy- and *N*-Methoxy-2-pyridones from the Insect Pathogenic Fungus *Cordyceps nipponica*

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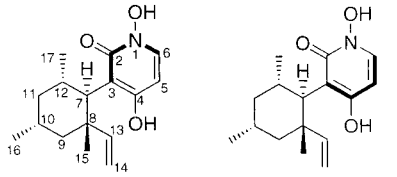
Received January 23, 2001

Bioassay-guided fractionation of the extracts from the insect pathogenic fungus *Cordyceps nipponica* BCC 1389 led to the isolation of *N*-hydroxy- and *N*-methoxy-2-pyridones, cordypyridones A−D (**1**−**4**). Structures of these compounds, including absolute configuration, were determined by spectroscopic methods, chemical conversions and single-crystal X-ray diffraction analyses. Codypyridones A and B, atropisomers of each other, exhibited potent in vitro antimalarial activity with IC_{50} values of 0.066 and 0.037 μg/mL, respectively, while their cytotoxicity was much weaker.

Malaria is by far the world's most important tropical parasitic disease, and the mortality is estimated to be over 1 million deaths each year. Because of the worsening problems in many parts of the world,[1] it is important to attend to the urgent need for new drugs, both from synthetic and natural products.[2] As part of our continuous search for novel bioactive compounds from microbial sources,[3] we have been screening a number of fungal cultures for in vitro antimalarial activity. One of the samples, an extract from culture broth of the insect pathogenic fungus *Cordyceps nipponica* BCC 1389 was shown to exhibit activity against *Plasmodium falciparum* (K1, multidrug resistant strain) with an IC_{50} value of 0.1 μg/mL. Therefore, we had undertaken the investigation of the chemical constituents of this fungus, and the study has led to the identification of four compounds, namely cordypyridones A−D (**1**−**4**). This paper describes the isolation, structure determination, and evaluation of the biological activities of these compounds.

Results and Discussion

The major secondary metabolite, cordypyridone A (**1**), was isolated as colorless crystals. The molecular formula of $C_{16}H_{23}NO_3$ was determined by elemental analysis, HRMS, and ^{1}H and ^{13}C NMR. NMR analyses (^{1}H, ^{13}C, DEPTs, COSY, HMQC, and HMBC) revealed that this compound possessed a cyclohexane ring substituted with three methyl groups, a vinyl group, and a heteroaromatic ring. The partial structure of the heteroaromatic ring, with a required formula of $C_5H_4NO_3$, was elucidated by ^{13}C NMR chemical shifts of the five aromatic carbons, and HMBC correlations (Figure 1), as 1,4-dihydroxy-2-pyridon-3-yl. The IR and UV spectra of cordypyridone A were in good agreement with this heteroaromatic subunit. The relative configuration of the cyclohexane substituents was determined by analysis of the NOESY spectrum (Figure 2) and the observation of four sets of 1,2-diaxial H−H couplings in the ^{1}H NMR spectrum with the *J*-values of 11.4−13.1 Hz. The equatorial orientations of the pyridone moiety, vinyl, and two methyl groups (C-16 and C-17), and the axial orientation of the C-15 methyl substituent, were consequently established. As shown in Figure 2, the conformation of the vinyl substituent was vertical to the cyclohexane ring and parallel to the heteroaromatic ring.



1

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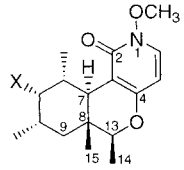
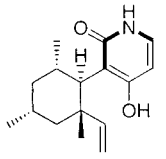


3 : X = H
4 : X = OH



5

Treatment of **1** with $LiAlH_4$ in THF gave a compound whose molecular mass ion $[M + H]^+$ is 16 unit less than

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(1) (a) Morel, C. M. *Parasitol. Today* **2000**, *16*, 522. (b) World Health Organization. WHO Fact Sheet No 94.

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(3) (a) Isaka, M.; Punya, J.; Lertwerawat, Y.; Tanticharoen, M.; Thebtaranonth, Y. *J. Nat. Prod.* **1999**, *62*, 329. (b) Isaka, M.; Jaturapat, A.; Kladwang, W.; Punya, J.; Lertwerawat, Y.; Tanticharoen, M.; Thebtaranonth, Y. *Planta Med.* **2000**, *66*, 473. (c) Isaka, M.; Tanticharoen, M.; Thebtaranonth, Y. *Tetrahedron Lett.* **2000**, *41*, 1657.

10.1021/jo0100906 CCC: $20.00 © 2001 American Chemical Society
Published on Web 06/09/2001

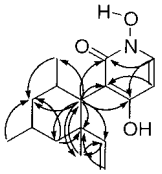


Figure 1. Selected HMBC correlations of **1**.

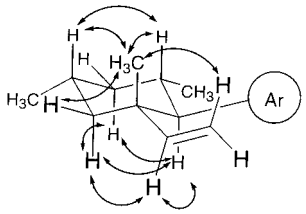


Figure 2. Selected NOESY correlations of **1**.

that of **1**. NMR spectra (^{1}H, ^{13}C) of this product were similar to those of **1**, and its IR spectrum was close to that of 4-hydroxy-2-pyridone (2,4-dihydroxypyridine), indicating the structure depicted as **5**. This result strongly supported the *N*-hydroxypyridone substructure elucidated for **1**.

The minor isomer, cordypyridone B (**2**), possessed the same molecular formula ($C_{16}H_{23}NO_3$) with cordypyridone A as determined by HRMS and ^{1}H and ^{13}C NMR spectra. IR, UV, and ^{1}H and ^{13}C NMR spectra of **2** were similar to those of **1**, except for the difference of their protons chemical shifts of H-7 (**1**, δ_H 2.61; **2**, δ_H 2.89) and H-11 (**1**, δ_H 2.96; **2**, δ_H 2.66). Detailed analyses of the NMR spectra (^{1}H, ^{13}C, DEPTs, COSY, HMQC, and HMBC) led to the elucidation of the structure analogous to that of **1**. However, a clear physicochemical difference between the two was realized upon examination of their specific rotations: **1**, $[\alpha]^{25}_D$ −11° (*c* 0.50, MeOH); **2**, $[\alpha]^{26}_D$ +164° (*c* 0.14, MeOH). These data suggested an atropisomeric relationship between these two compounds.

Heating a solution of **1** in dioxane at 90 °C for 40 h gave a 65:35 mixture of **1** and **2** (determined by ^{1}H NMR). Each compound was separated by preparative reversed-phase HPLC. The optical rotation value of **2** obtained from this thermal conversion, $[\alpha]^{27}_D$ +162° (*c* 0.11, MeOH), was consistent to that of the naturally occurring sample. Under the same thermal conditions, compound **2** gave a mixture of **1**/**2** = 64:36. On the basis of this experimental evidence, it was concluded that compounds **1** and **2** are atropisomers to each other that can interconvert in solution at high temperature. The atropisomerization was undetectable during the isolation process of these compounds at room temperature. Heating the solution of **1** in MeOH-d_4 at 60 °C resulted in slow interconversion to give **1**/**2** = 58:42 at 20 h, 49:51 at 40 h, 43:57 at 85 h, and 42:58 at 160 h. Thus, the atropisomerization equilibrium ratio is slightly different from that observed in dioxane.

Extensive trials to obtain suitable crystals for X-ray analysis had been unsuccessful; compound **1** crystallized as thin plates. However, crystallization of the minor isomer **2** from acetone gave prisms, one of which was subjected to X-ray crystallographic analysis. The C-3−C-7 stereochemistry of **2** was thus proven which, in turn, determined the stereostructure of the major metabolite **1** as depicted. The relative configuration on the cyclohexane ring as well as the conformation, elucidated by NMR analyses, was also confirmed by X-ray crystallographic results.[4]

Cordypyridone C (**3**) was isolated in very low yield (2.2 mg of pure compound from 15 L of culture broth). Comparison of ^{1}H NMR spectra of **1** and **3** indicated the absence of the vinyl protons (H-13 and H-14; 3H in **1**), and the appearance of a low-field methine proton (δ_H 4.10, d, J = 6.5 Hz; δ_C 86.6), a methyl group (3H, δ_H 1.18, d, J = 6.5 Hz; δ_C 86.6) adjacent to this methine, and an oxymethyl group (δ_H 3.92, 3H, s; δ_C 64.3, q). The molecular formula of $C_{17}H_{25}NO_3$ was determined by HRMS and NMR. A NOESY cross-peak observed between the oxymethyl group and H-6 indicated that the hydroxyl group attached to nitrogen atom was methylated. A tricyclic structure as depicted in **3** was proposed from these data. Detailed analysis of the NOESY spectrum and H−H coupling constants of the cyclohexane protons revealed that this compound has the same relative configurations as **1** and **2**. The configuration at C-13 was revealed by the NOESY correlations; H-13 to H-7 and axial H-9, the methyl protons (H-14) to equatorial H-9 and the tertiary methyl group (H-15). The proton signal of the axial methyl (H-15, 0.65 ppm) was high-field shifted when compared to that of compound **1** (H-15, 1.01 ppm), probably due to the shielding effect by the heteroaromatic ring.

Cordypyridone D (**4**) had a molecular formula of $C_{17}H_{25}NO_4$. 1D- and 2D-NMR analyses revealed the structure of this compound as the 11-hydroxy analogue of **3**. The methine proton, H-11, appeared as a broad singlet. The small $J_{11,12}$-value of 2.9 Hz indicated that H-11 must be placed in the equatorial position, hence, the axial orientation of the hydroxyl group.

Due to the fact that the available data obtained for compounds **3** and **4** could not rule out the alternative 4-pyridone structural skeleton (depicted as **6**) and also the absolute configuration of cordypyridones A−D (**1**−**4**) was not clear, we addressed these uncertainties by chemical means. Treatment of **1** with MeI (1 equiv)/K_2CO_3 in 2-butanone at room temperature gave a monomethylated product **7** (58%) as a ca. 8:1 mixture of atropisomers, a dimethoxy derivative **8** (21%; a ca. 10:1 mixture of atropisomers), and unreacted **1** (15%). It was noticed that the monomethyl analogue **7** slowly underwent atropisomerization even at room temperature. Compound **7** was treated with $Hg(OAc)_2$ in MeOH-d_4, and the reaction was monitored by ^{1}H NMR. After standing at room temperature for 24 h, the protons signals of the vinyl group had completely disappeared. Signals at δ_H 4.37 (1H, dd, J = 9.7, 4.8 Hz) and 2.15−2.05 (2H) suggested that the expected oxymercuration reaction took place. The high-field shift of the C-15 methyl signal from δ_H 1.13 to 0.77 was also in good agreement with the tricyclic structure **9** (δ_H 0.65 for **3** in acetone-d_6). However, isolation of an organomercurium after treatment with sat. NaCl (**9**; X = Cl), with the intent of X-ray diffraction analysis of the

(4) The heteroaromatic moieties of cordypyridones A and B may be assigned as tautomeric between the 1,4-dihydroxy-2-pyridone (depicted as **1** and **2**) and the 2,4-dihydroxypyridine *N*-oxide forms. The C(4)−O bond length of 1.349 Å, determined by a single crystal of cordypyridone B, was close to that of the phenol, while the C(2)−O bond (1.257 Å) was much shorter. Slightly shorter C(2)−O bond length (1.235 Å) observed for the 2-pyridone **12** (see text below) suggested a major contribution of the 1,4-dihydroxy-2-pyridone structure, rather than 2,4-dihydroxypyridine *N*-oxide, in the tautomerism of cordypyridones A and B. Also the N−O bond length of cordypyridone B (1.380 Å) was close to that of **12** (1.395 Å).

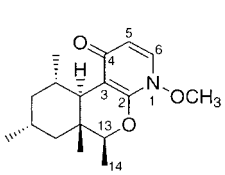
Table 1. ^{1}H NMR Data of Cordypyridones A−D (acetone-d_6)

position	1	2	3	4
5	6.03 (d, 7.7)	6.01 (d, 7.6)	5.73 (d, 7.6)	5.74 (d, 7.6)
6	7.66 (d, 7.7)	7.61 (d, 7.6)	7.57 (d, 7.6)	7.56 (d, 7.8)
7	2.61 (d, 11.4)	2.89 (d, 11.7)	1.97 (d, 11.3)	2.39 (d, 12.0)
9	1.11 (dd, 12.9, 12.5)	1.14(dd, 12.9, 12.4)	0.68 (dd, 12.7, 12.7)	1.39 (dd, 12.7, 12.6)
	1.32 (ddd, 12.6, 2.8, 2.5)	1.30 (ddd, 12.4, 2.6, 2.5)	1.60 (ddd, 12.6, 2.9, 2.5)	1.18 (dd, 12.8, 3.3)
10	1.78 (m)	1.78 (m)	1.63 (m)	1.76 (m)
11	0.63 (ddd, 13.1, 13.1, 12.3)	0.69 (ddd, 13.0, 13.0, 12.6)	0.60 (ddd, 12.7, 12.3, 12.3)	3.52 (brs)
1.75 (m)	1.75 (m)	1.78 (dddd, 13.0, 2.8, 2.7, 2.5)		
12	2.96 (m)	2.66 (m)	2.61 (m)	2.58 (dqd, 12.0, 6.1, 2.9)
13	5.90 (dd, 17.6, 10.8)	5.99 (dd, 17.2, 10.5)	4.10 (q, 6.5)	4.13 (q, 6.5)
14	4.62 (dd, 10.8, 1.5)	4.51 (dd, 10.6, 1.4)	1.18 (d, 6.5)	1.17 (d, 6.4)
	4.67 (dd, 17.6, 1.5)	4.63 (dd, 17.3, 1.3)		
15	1.01 (s)	1.08 (s)	0.65 (s)	0.65 (s)
16	0.88 (d, 6.4)	0.88 (d, 6.3)	0.88 (d, 6.3)	0.97 (d, 6.8)
17	0.59 (d, 6.4)	0.68 (d, 6.4)	1.10 (d, 5.7)	1.22 (d, 6.1)
1-OCH_3	3.91 (s)	3.92 (s)		

Table 2. ^{13}C NMR Data of Compounds 1−4 (acetone-d_6)

position	1	2	3	4
2	159.9 (s)	161.1 (s)	158.3 (s)	158.4 (s)
3	112.0 (s)	112.3 (s)	110.2 (s)	110.8 (s)
4	163.4 (s)	162.2 (s)	163.9 (s)	164.3 (s)
5	98.0 (d)	98.2 (d)	98.7 (d)	98.9 (d)
6	131.3 (d)	129.8 (d)	134.5 (d)	134.4 (d)
7	50.0 (d)	51.1 (d)	50.3 (d)	44.1 (d)
8	44.6 (s)	44.8 (s)	37.2 (s)	37.1 (s)
9	50.0 (t)	50.3 (t)	46.1 (t)	38.1 (t)
10	28.5 (d)	28.6 (d)	26.8 (d)	32.2 (d)
11	46.1 (t)	46.1 (t)	45.9 (t)	74.6 (d)
12	28.7 (d)	29.4 (d)	28.7 (d)	34.2 (d)
13	151.0 (d)	151.1 (d)	86.6 (d)	86.9 (d)
14	109.3 (t)	109.0 (t)	16.0 (q)	16.1 (q)
15	20.1 (q)	19.4 (q)	14.8 (q)	14.8 (q)
16	23.2 (q)	23.2 (q)	23.1 (q)	19.3 (q)
17	21.7 (q)	21.5 (q)	25.6 (q)	20.0 (q)
1-OCH_3	-	-	64.3 (q)	64.4 (q)

heavy atom-containing molecule, was unsuccessful, and only **7** was recovered after workup. The in situ reduction by addition of $NaBH_4$/aq NaOH or $NaBH_4$/H_2O into the oxymercuration intermediate also failed to give any product apart from the recovered **7**.



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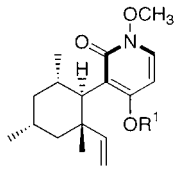
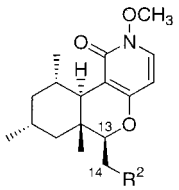
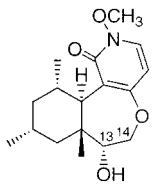


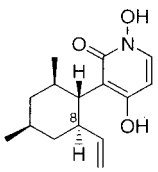
7 : R^1 = H
8 : R^1 = CH_3



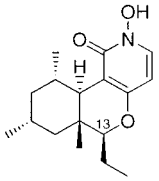
9 : R^2 = HgX
10 : R^2 = OH
12 : R^2 = *p*-bromobenzoyloxy



11



13
(relative configuration)



14
(relative configuration)

The alternative, stepwise approach involving epoxidation of **7** followed by cyclization proved fruitful. This strategy was based on our hypothesis that the reaction at the olefinic π-face opposite to the heteroaromatic ring (see Figure 2) should be nicely set up for exocyclization to form the corresponding pyran ring with the correct C-13 configuration. Thus, treatment of **7** with a slight excess *m*-CPBA in $CDCl_3$ at room-temperature resulted in slow epoxidation as monitored by ^{1}H NMR. After complete disappearance of the vinyl protons signals, the $CDCl_3$ solution was concentrated, diluted with EtOAc, washed with aq K_2CO_3, and concentrated to obtain the crude epoxide. This was, without purification, treated with K_2CO_3 (s) in DMF (rt, 4 days). The desired cyclization product **10** was obtained as a major product together with small amount of an endocyclization product **11**. The purified major product **10** was converted into the corresponding *p*-bromobenzoate **12** under standard reaction conditions. The absolute configuration of compound **12** was established, as depicted, using anomalous scattering X-ray crystallographic methods. This, in turn, established the absolute configuration of **1** and **2**. The ^{1}H and ^{13}C NMR chemical shifts of the alcohol **10** were very similar to those of cordypyridone C (**3**), particularly the signals of the heteroaromatic ring, except for the H-14 and C-14 signals. The IR and UV spectra of **10** were also similar to those of **3** except for the appearance of a hydroxyl absorption (ν 3421 cm^{-1}) in IR. The optical rotation values of both **10** ($[\alpha]^{27}_D$ +152°, *c* 0.15, MeOH) and **3** ($[\alpha]^{24}_D$ +243°, *c* 0.06, MeOH) together with the described data indicated that the cyclization product **10** was the 14-hydroxy analogue of cordypyridone C. Thus, cordypyridone C exists as the 1-methoxy-2-pyridone structure depicted as **3** and not the 1-methoxy-4-pyridone (**6**). The absolute configuration of **3** should be identical to that of **1**. By analogy, cordypyridone D should also possess the 1-methoxy-2-pyridone moiety with the same absolute configuration as compounds **1**−**3**. The structure of the minor endocyclization product **11**, bearing the same molecular formula as **10** (HRMS), was elucidated by 1D- and 2D-NMR analyses, and the (13*R*)-configuration was established.[5]

(5) Structure elucidation of compound **11** is detailed in the Supporting Information.

Table 3. Antimalarial Activity and Cytotoxicity of Cordypyridones A−D, and Compounds 5 and 8

compound	antimalarial activity (IC_{50}, μg/mL) *P. falciparum* K1	cytotoxicity (IC_{50}, μg/mL) KB cells	BC-1 cells	Vero cells
cordypyridone A (**1**)	0.066	15.7	3.9	6.3
cordypyridone B (**2**)	0.037	8.4	3.7	5.3
cordypyridone C (**3**)	>20	*c*	*c*	*c*
cordypyridone D (**4**)	7.8	>20	>20	>20
compound **5**	>20	>20	>20	>20
compound **8**[a]	14	>20	>20	15
chloroquine diphosphate[b]	0.16	16	>20	>20
artemisinin[b]	0.0011	>20	>20	>20

[a] A 11:1 mixture of atropisomers. [b] Standard antimalarial compounds. [c] Cytotoxicity of compound **3** was not tested due to the small sample amount.

The structure of cordypyridones A and B are closely related to pyridoxatin (**13**) which was previously isolated from *Acremonium* sp. BX86.[6] Pyridoxatin is reported to be present in solution as a mixture of rotamers, and the relative configuration of this compound had been determined by NMR analyses. The compound has also been isolated by Jegorov et al as tolypocin [HL] from *Tolypocladium geodes*.[7] They also isolated the iron(III) trischelate complex of this ligand [FeL_3] (named, terricolin) from *T. terricola*.[7] It is interesting to note that the absolute configuration of the ligand elucidated from the X-ray crystallographic analysis (anomalous scattering methods) and CD spectra, depicted as **13**, is opposite to that of cordypyridones. Most recently, the 8-methyl analogue of pyridoxatin was isolated from an unidentified fungus OS-F61800.[8] By comparison of the spectral data and optical rotation (lit.[8] $[\alpha]^{23}_D$ −9.4°, *c* 0.43, MeOH), we believe that this compound is identical to cordypyridone A.

Cordypyridone C (**3**) has similar structure to fusaricide (**14**) which was recently isolated from *Fusarium* sp.[9] The relative configuration of cordypyridone C is analogous to that of fusaricide. The close optical rotation values between **3** and fusaricide (lit.[9] $[\alpha]_D$ +194°, *c* 0.12, $CHCl_3$) suggested that these compounds may have identical absolute configuration.

Cordypyridones A−D (**1**−**4**) and the semisynthetic analogues **5** and **8** were subjected to in vitro antimalarial activity screening. For comparison, the cytotoxicities of these compounds against two cancer cells (KB, BC-1) and Vero cells (African green monkey kidney fibroblast) were determined. Cordypyridones A and B exhibited significant activity against the malaria parasite (*P. falciparum* K1), while other derivatives, **3**, **4**, **5**, and **8**, showed weak activity or were inactive up to a concentration of 20 μg/mL (Table 3). Comparison of the results of **1** and **5**, and the weak antimalarial activity of **8** suggested that the hydroxyl group on nitrogen is indispensable for the antimalarial activity of compound **1** (and **2**). The mechanism of the parasite growth inhibition by these compounds may be due to their iron chelating ability by analogy to other hydroxamic acid antimalarials.[10] Compounds **1** and **2** also showed cytotoxicity, but the magnitude of activity was much lower than their antimalarial activity. Although the antimalarial activities of cordypyridones A and B are much weaker than known drugs, such as artemisinin, the novelty of the chemical skeleton and their good selectivity index deserve further investigation.

Experimental Section

Fermentation, Extraction, and Isolation. *C. nipponica* was collected from Khao Yai National Park in Central Thailand, on Neuroptera-larva, and identified by Dr. Nigel L. Hywel-Jones of the Mycology Research Unit, BIOTEC. The fungus is deposited at the BIOTEC Culture Collection as BCC 1389. A culture maintained on a potato dextrose agar (30 °C, 8 days) was inoculated into 60 × 1 L Erlenmeyer flasks each containing 250 mL of minimum salt medium and statically incubated at 22 °C for 29 days. The flask cultures were separated by filtration into supernatant (ca. 15 L) and wet mycelial cake. Supernatant was separated into several portions, and each portion was extracted three times with an equal volume of EtOAc. The combined EtOAc solution was partially concentrated into ca. 500 mL, dried over $MgSO_4$, and concentrated in vacuo to obtain a light brown amorphous solid (1.2 g). The crude extract was fractionated by silica gel column chromatography with MeOH/CH_2Cl_2 as eluent (gradient elution; MeOH/CH_2Cl_2 = 2:98, 5:95 then 10:90). Fractions eluted with MeOH/CH_2Cl_2 = 5:95 contained a mixture of cordypyridones. The mixture was passed through a Sephadex LH-20 column with MeOH as eluent. Compounds **3** and **4** were included in earlier elute (84 mg), where a mixture containing predominantly **1** and **2** (166 mg) was obtained from the latter elute. Compounds **3** and **4** were separated by preparative HPLC using a reversed-phase column (Prep Nova-Pak HR C_{18}, 6 μm, 40 × 100 mm) with MeCN/H_2O = 30:70 as eluent at a flow rate of 20 mL/min: **3**, t_R 21 min, 11 mg; **4**, t_R 14 min, 2.2 mg). Separation of compounds **1** and **2** was also established by repeated preparative HPLC (MeCN/H_2O = 45:55): **1**, t_R 18 min, 85 mg; **2**, t_R 13 min, 11 mg).

Compounds **1** and **2** were isolated also from a MeOH extract of mycelia. To the MeOH extract (ca. 2 L) was added H_2O (100 mL), and washed with hexane (1.2 L). The aqueous MeOH layer was partially concentrated under reduced pressure at room temperature to ca. 150 mL. H_2O (300 mL) was added to the mixture, extracted with EtOAc (2 × 500 mL), and concentrated to obtain a light brown solid (0.44 g). Partial purification by Sephadex LH-20 chromatography, followed by separation by repeated preparative HPLC, gave pure compounds **1** (109 mg) and **2** (15 mg).

Cordypyridone A (8-Methyl-pyridoxatin) (1). Colorless crystals; mp 261−263 °C (dec); $[\alpha]^{25}_D$ −11° (*c* 0.50, MeOH); UV (MeOH) λ_{max} (log ϵ) 213 (4.48), 247 (3.68), 287 (3.60) nm; IR (KBr) ν_{max} 3090, 1632, 1542, 1434, 1267, 1043, 802 cm^{-1}; MS (EI) *m/z* 277 $[M]^+$, 260, 208, 164, 150, 137, 124; HRMS (ESI-TOF) *m/z* $[M + H]^+$ 278.1747 (calcd for $C_{16}H_{24}NO_3$, 278.1756). Anal. Found: C 69.30%, H 8.10%, N 5.15%. Calcd for $C_{16}H_{23}NO_3$: C 69.29%, H 8.36%, N 5.05%.

Cordypyridone B (2). Colorless crystals; mp 252−255 °C (dec); $[\alpha]^{26}_D$ +164° (*c* 0.14, MeOH); UV (MeOH) λ_{max} (log ϵ) 212 (4.54), 246 (3.61), 288 (3.72) nm; IR (KBr) ν_{max} 3083, 1636, 1538, 1440, 1267, 1233, 1057, 799 cm^{-1}; MS (EI) *m/z* 277 $[M]^+$, 260, 208, 164, 150, 137, 124; HRMS (ESI-TOF) *m/z* $[M + H]^+$ 278.1769 (calcd for $C_{16}H_{24}NO_3$, 278.1756).

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Cordypyridone C (3). Colorless crystals; mp 150−151 °C (dec); $[\alpha]^{24}_D$ +243° (*c* 0.06, MeOH); UV (MeOH) λ_{max} (log ϵ) 218 (4.41), 292 (3.67) nm; IR (KBr) ν_{max} 1639, 1599, 1538, 1457, 1228, 992 cm^{-1}; MS (ESI-TOF) *m/z* 314 $[M + Na]^+$, 292 $[M + H]^+$, 223, 218; HRMS (ESI-TOF) *m/z* $[M + H]^+$ 292.1924 (calcd for $C_{17}H_{26}NO_3$, 292.1912).

Cordypyridone D (4). Colorless crystals; mp 206−209 °C (dec); $[\alpha]^{26}_D$ +249° (*c* 0.14, MeOH); UV (MeOH) λ_{max} (log ϵ) 218 (4.47), 293 (3.75) nm; IR (KBr) ν_{max} 3488, 1631, 1585, 1536, 1450, 991 cm^{-1}; MS (EI) *m/z* 307 $[M]^+$, 276, 258, 150; HRMS (ESI-TOF) *m/z* $[M + H]^+$ 308.1859 (calcd for $C_{17}H_{26}NO_4$, 308.1862).

$LiAlH_4$ Reduction of 1. To a cold solution of **1** (20 mg) in THF (1 mL) was added $LiAlH_4$ (20 mg), and the mixture was stirred at room temperature for 12 h. After usual aqueous workup, the crude mixture (9.8 mg) was purified by preparative HPLC to obtain compound **5** (9.0 mg).

1-Dehydroxycordypyridone A (5). Colorless crystals: mp 289−291 °C (dec); IR (KBr) ν_{max} 3133, 2949, 1615, 1553, 1463, 1441, 1318, 1301, 1272, 1030, 909, 804, 605 cm^{-1}; HRMS (ESI-TOF) *m/z* $[M + H]^+$ 262.1790 (calcd for $C_{16}H_{24}NO_2$, 262.1807); ^{1}H NMR (acetone-d_6, 400 MHz) δ 7.13 (1H, d, *J* = 7.1 Hz, H-6), 5.97 (1H, dd, *J* = 17.5, 10.7 Hz, H-14), 5.89 (1H, d, *J* = 7.1 Hz, H-5), 4.72 (1H, dd, *J* = 17.6, 1.5 Hz, H-14a), 4.64 (1H, dd, *J* = 10.7, 1.5 Hz, H-14b), 3.04 (1H, m, H-12), 2.58 (1H, d, *J* = 11.2 Hz, H-7), 1.79 (1H, m, H-10), 1.76 (1H, m, H-11a), 1.32 (1H, brd, *J* = ca13 Hz, H-9a), 1.11 (1H, m, H-9b), 1.11 (3H, s, H-15), 0.88 (3H, d, *J* = 6.2 Hz, H-16), 0.67 (3H, d, *J* = 6.4 Hz, H-17), 0.63 (1H, ddd, *J* = 12.3, 12.3, 12.3 Hz, H-11b); ^{13}C NMR (acetone-d_6, 100 MHz) δ 165.3 (s, C-2), 165.3 (s, C-4), 151.6 (d, C-13), 133.3 (d, C-6), 112.4 (s, C-3), 109.0 (t, C-14), 99.8 (d, C-5), 50.3 (t, C-9), 49.5 (d, C-7), 46.3 (t, C-12), 44.6 (s, C-8), 28.8 (d, C-12), 28.6 (d, C-10), 23.3 (q, C-16), 20.3 (q, C-15), 21.9 (q, C-17).

Single-Crystal X-ray Diffraction Analysis of Cordypyridone B (2). Colorless crystals of **2** were obtained by recrystallization from acetone. Crystallographic data: $C_{16}H_{23}NO_2$, MW 261.37, Monoclinic, $P2_1$, *a* = 13.072(2) Å, *b* = 9.820(1) Å, *c* = 13.352(1) Å, and β = 112.31(1)°, *V* = 1585.7(2) Å^3, *Z* = 4, μ = 0.071 mm^{-1}, D_x = 1.095 g/cm^{-3}, *R* = 0.0591 (R_w = 0.1413 for 1715 observed reflections). Atomic coordinates, bond lengths, bond angles, and thermal parameters have been deposited with the Cambridge Crystallographic Data Center (CCDC 151697). Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK (fax: +44-(0)1223−336033 or e-mail: deposit@ccdc.cam.ac.uk).

Methylation of 1. To a solution of **1** (27.7 mg, 0.10 mmol) in 2-butanone (0.5 mL) were added K_2CO_3 (s) (ca. 100 mg) and MeI (200 μL of a 0.50 M solution in 2-butanone, 0.10 mmol), and the mixture was stirred at room temperature for 20 h. After usual aqueous workup, the crude solid (30 mg) was subjected to preparative HPLC (MeCN/H_2O = 40:60) to obtain a monomethyl derivative **7** (16.8 mg, 58% yield), a dimethyl derivative **8** (6.3 mg, 21%), and recovered starting material **1** (2.5 mg, 15%).

1-*O*-Methylcordypyridone A (7). Colorless crystals: MS (ESI-TOF) *m/z* 314 $[M + Na]^+$, 292 $[M + H]^+$; ^{1}H NMR (acetone-d_6, 400 MHz) δ 7.49 (1H, d, *J* = 7.8 Hz, H-6), 5.97 (1H, dd, *J* = 17.7, 10.8 Hz, H-13), 5.90 (1H, d, *J* = 7.8 Hz, H-5), 4.71 (1H, dd, *J* = 17.6, 1.6 Hz, H-14a), 4.64 (1H, dd, *J* = 10.7, 1.6 Hz, H-14b), 3.90 (3H, s, 1-OCH_3), 3.02 (1H, m, H-12), 2.61 (1H, d, *J* = 11.2 Hz, H-7), 1.79 (1H, m, H-10), 1.75 (1H, m, H-11a), 1.32 (1H, ddd, *J* = 12.7, 2.6, 2.6 Hz, H-9a), 1.12 (1H, dd, *J* = 12.4, 12.4 Hz, H-9b), 1.11 (3H, s, H-15), 0.88 (3H, d, *J* = 6.3 Hz, H-16), 0.66 (3H, d, *J* = 6.5 Hz, H-17), 0.64 (1H, ddd, *J* = 12.4, 12.4, 12.4 Hz, H-11b): following proton signals due to the atropisomer were detected; 7.44 (1H, d, *J* = 7.8 Hz, H-6), 6.03 (1H, m, H-14), 5.86 (1H, d, *J* = 7.8 Hz, H-5), 4.63 (1H, m, H-14a), 4.60 (1H, dd, *J* = 10.8, 2.0 Hz, H-14b), 3.88 (3H, s, 1-OCH_3), 2.96 (1H, d, *J* = 11.7 Hz, H-7), 2.68 (1H, m, H-12), 1.08 (3H, s, H-15), 0.70 (3H, d, *J* = 6.5 Hz, H-17).

1-*O*-4-*O*-Dimethylcordypyridone A (8). Colorless crystals: mp 97−99 °C; IR (KBr) ν_{max} 2946, 1649, 1592, 1526, 1459, 1085, 1004, 765 cm^{-1}; HRMS (ESI-TOF) *m/z* $[M + H]^+$ 306.2056 (calcd for $C_{18}H_{27}NO_3$, 306.2069); ^{1}H NMR (acetone-d_6, 400 MHz) δ 7.69 (1H, d, *J* = 8.0 Hz, H-6), 6.12 (1H, d, *J* = 8.0 Hz, H-5), 5.78 (1H, m, H-13), 4.65 (1H, dd, *J* = 11.1, 1.4 Hz, H-14a), 4.63 (1H, dd, *J* = 17.3, 1.5 Hz, H-14b), 3.93 (3H, s, 1-OCH_3), 3.79 (3H, s, 4-OCH_3), 3.05 (1H, m, H-12), 2.59 (1H, d, *J* = 11.3 Hz, H-7), 1.78 (1H, m, H-10), 1.75 (1H, m, H-11a), 1.33 (1H, ddd, *J* = 12.7, 2.7, 2.5 Hz, H-9a), 1.09 (1H, dd, *J* = 12.6, 12.6 Hz, H-9b), 1.06 (3H, s, H-15), 0.88 (3H, d, *J* = 6.3 Hz, H-16), 0.63 (3H, d, *J* = 6.5 Hz, H-17), 0.62 (1H, ddd, *J* = 13.0, 12.3, 12.2 Hz, H-11b); ^{13}C NMR (acetone-d_6, 100 MHz) δ 165.3 (s, C-4), 158.9 (s, C-2), 151.1 (d, C-13), 134.8 (d, C-6), 116.3 (s, C-3), 108.8 (t, C-14), 93.6 (d, C-5), 64.3 (q, 1-OCH_3), 56.4 (q, 4-OCH_3), 50.2 (t, C-9), 50.1 (d, C-7), 46.1 (t, C-12), 44.6 (s, C-8), 28.7 (d, C-12), 28.5 (d, C-10), 23.2 (q, C-16), 21.9 (q, C-17), 20.0 (q, C-15).

Epoxidation of 7 and Cyclization. To a $CDCl_3$ (0.3 mL) solution of compound **6** (10.8 mg, 37 μmol) in an NMR sampling tube was added a $CDCl_3$ (0.3 mL) solution of slight excess *m*-chloroperbenzoic acid (70 wt %, 10 mg), and the reaction was monitored by ^{1}H NMR. After disappearance of the ^{1}H NMR signals due to the olefinic protons of **7** (room temp, 11 h), the solution was concentrated and diluted with EtOAc, washed with sat. $NaHCO_3$, dried over $MgSO_4$, and concentrated in vacuo to obtain a crude sample (11.5 mg) mainly containing the corresponding epoxide. The crude sample, without purification, was dissolved in DMF (0.5 mL) and K_2CO_3 (100 mg) was added and stirred for 3 days. After aqueous workup, the crude mixture was subjected to prep HPLC to obtain compounds **10** (3.3 mg) and **11** (0.8 mg).

14-Hydroxycordypyridone C (10). Colorless amorphous solid: $[\alpha]^{27}_D$ +152° (*c* 0.15, MeOH); UV (MeOH) λ_{max} (log ϵ) 218 (4.39), 292 (3.67) nm; IR (KBr) ν_{max} 3421, 1637, 1594, 1541, 1456, 1226, 1060, 996 cm^{-1}; HRMS (ESI-TOF) *m/z* $[M + H]^+$ 308.1858 (calcd for $C_{17}H_{26}NO_4$, 308.1862); ^{1}H NMR (acetone-d_6, 400 MHz) δ 7.59 (1H, d, *J* = 7.9 Hz, H-6), 5.81 (1H, d, *J* = 7.9 Hz, H-5), 4.00 (1H, dd, *J* = 7.9, 2.9 Hz, H-13), 3.92 (3H, s, OCH_3), 3.88 (1H, brs, O*H*), 3.74 (1H, dd, *J* = 11.9, 2.8 Hz, H-14a), 3.54 (1H, dd, *J* = 11.9, 8.0 Hz, H-14b), 2.65 (1H, m, H-12), 1.96 (1H, d, *J* = 11.3 Hz, H-7), 1.78 (2H, m, H-9a and H-11a), 1.63 (1H, m, H-10), 1.10 (3H, d, *J* = 5.6 Hz, H-17), 0.88 (3H, d, *J* = 6.5 Hz, H-16), 0.85 (1H, dd, *J* = 12.4, 12.4 Hz, H-9b), 0.68 (3H, s, H-15), 0.63 (1H, ddd, *J* = 12.5, 12.4, 12.4 Hz, H-11b); ^{13}C NMR (acetone-d_6, 100 MHz) δ 164.0 (s, C-4), 158.1 (s, C-2), 134.7 (d, C-6), 111.4 (s, C-3), 98.9 (d, C-5), 91.6 (d, C-13), 64.3 (q, 1-OCH_3), 62.6 (t, C-14), 49.9 (d, C-7), 46.7 (t, C-9), 45.8 (t, C-11), 37.5 (s, C-8), 28.2 (d, C-12), 26.7 (d, C-10), 25.4 (q, C-17), 23.0 (q, C-16), 15.6 (q, C-15).

14-(*p*-Bromobenzoyloxy)cordypyridone C (12). Compound **10** (3.3 mg) was converted into *p*-bromobenzoate **12** following standard procedure (*p*-bromobenzoyl chloride, pyridine), and purified by prep HPLC (yield, 4.2 mg). Recrystallization from MeOH gave colorless needles: mp 180−181 °C; MS (ESI-TOF) *m/z* 490 and 492 $[M + H]^+$; ^{1}H NMR (acetone-d_6, 400 MHz) δ 7.98 (2H, d, *J* = 8.5 Hz, *p*-bromobenzoyl), 7.74 (2H, d, *J* = 8.5 Hz, *p*-bromobenzoyl), 7.62 (1H, d, *J* = 7.6 Hz, H-6), 5.79 (1H, d, *J* = 7.7 Hz, H-5), 4.61 (1H, m, H-13a), 4.37−4.39 (2H, m, H-13b and H-14), 3.94 (3H, s, OCH_3), 2.69 (1H, m, H-12), 2.06 (1H, overlapped with solvent signal, H-7), 1.88 (1H, ddd, *J* = 12.5, 2.5, 2.4 Hz, H-9a), 1.82 (1H, m, H-11a), 1.69 (1H, m, H-10), 1.12 (3H, d, *J* = 5.8 Hz, H-17), 0.97 (1H, dd, *J* = 12.6, 12.3 Hz, H-9b), 0.92 (3H, d, *J* = 6.4 Hz, H-16), 0.81 (3H, s, H-15), 0.67 (1H, ddd, *J* = 12.7, 12.4, 12.3 Hz, H-11b); ^{13}C NMR (acetone-d_6, 100 MHz) δ 166.2 (s), 163.7 (s), 158.2 (s), 135.0 (d), 132.8 (d), 130.2 (d), 130.3 (s), 128.5 (s), 111.6 (s), 98.7 (d), 87.7 (d), 65.3 (t), 64.4 (q), 49.6 (d), 46.5 (t), 45.7 (t), 37.8 (s), 28.3 (d), 26.7 (d), 25.3 (q), 23.0 (q), 15.7 (q).

Single-Crystal X-ray Diffraction Analysis of 12. Crystallographic data: $C_{24}H_{28}NO_5Br$, MW 490.40, monoclinic, C2, *a* = 24.898(3) Å, *b* = 10.198(2) Å, *c* = 9.757(1) Å, and β = 108.84(1)°, *V* = 2344.8(5) Å^3, *Z* = 4, calculated density = 1.389 g/cm^{-3}, *R*-factor = 0.0804 (R_w = 0.1775 for 2379 observed reflections). Flack parameter = 0.00(2) [1.00(2) for inverted coordinates]. The absolute configuration of compound **12** was established using anomalous scattering methods. Atomic

coordinates, bond lengths, bond angles, and thermal parameters have been deposited with the Cambridge Crystallographic Data Center (CCDC 151698). Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK (fax: +44-(0)1223−336033 or e-mail: deposit@ccdc.cam.ac.uk).

Biological Assays. Assay for activity against *P. falciparum* (K1, multidrug resistant strain) was performed using the protocol previously reported[3] which follows the microculture radioisotope technique described by Desjardins.[11] IC_{50} represents the concentration that causes 50% reduction of parasite growth as indicated by the in vitro uptake of [^{3}H]-hypoxanthine by *P. falciparum*. Cytotoxicity of the purified compounds against human epidermoid carcinoma (KB), human breast cancer cells (BC-1) and Vero cells were tested using the colorimetric method.[12] IC_{50} values of a standard compound ellipticine are 0.46 μg/mL for KB cells, and 0.60 μg/mL for BC-1 cells in our system.

Acknowledgment. Financial support from the Biodiversity Research and Training Program (BRT) and the Thailand-Tropical Diseases Research Program (T-2) are gratefully acknowledged. We are also grateful to the BIOTEC's fermentation technology laboratory and screening laboratories for mass cultivation and bioassay, respectively. One of us (Y. T.) thanks NSTDA for the Senior Research Fellowship Award.

Supporting Information Available: ^{1}H and ^{13}C NMR spectra of compounds **1**−**4**, ORTEP diagrams of compounds **2** and **12**, structure elucidation and spectroscopic data of compound **11**. This material is available free of charge via the Internet at http://pubs.acs.org.

JO0100906

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