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STRUCTURE OF FISCHERIN, A NEW TOXIC METABOLITE FROM AN ASCOMYCETE, *NEOSARTORYA FISCHERI* VAR. *FISCHERI*[1,2]

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ABSTRACT.—A new toxic metabolite named fischerin [**1**] from an Ascomycete. *Neosartorya fischeri* var. *fischeri*, which caused lethal peritonitis in mice, was deduced to have a structure including a 1,4-dihydroxy-3,5-disubstituted-2(1*H*)-pyridone moiety by chemical and spectral data.

In our screening program on biologically active components of fungi, the Me_2CO extract of an Ascomycete, *Neosartorya fischeri* (Wehmer) Malloch & Cain var. *fischeri*, caused tremors and lethal peritonitis in mice. The tremorgenic principles of the fungus were proved to be identical with fumitremorgins A and B (1,2), which were formerly isolated from *Aspergillus fumigatus* (3,4). The toxic component of the fungus causing peritonitis was further isolated and named fischerin. This report deals with the isolation and structure determination of fischerin.

RESULTS AND DISCUSSION

The Me_2CO extract of *N. fischeri* var. *fischeri* cultured on sterilized rice was treated with *n*-hexane to remove the fatty portion. The defatted extract was then treated with $CHCl_3$ to obtain a $CHCl_3$-soluble portion. The $CHCl_3$-soluble portion (yield 0.28% from the rice medium) was divided into non-polar and polar fractions through chromatography on a Si gel column. From the non-polar fraction causing severe tremors in mice, fumitremorgins A and B (3,4) were isolated (1,2). On the other hand, the polar fraction causing lethal peritonitis in mice was separated on a Sephadex column, a flash chromatographic column of Si gel, and a medium pressure liquid chromatographic (mplc) column of reversed-phase octadecyl Si gel (ODS), successively, to afford fischerin [**1**] (yield 1.3% from the $CHCl_3$-soluble portion). Fischerin caused death after acute peritonitis in mice on ip administration of 100 mg/kg within 2 h.

Fischerin [**1**], pale yellow powder, was soluble in 5% $NaHCO_3$ and 1 N NaOH, insoluble in 1 N HCl, and positive to the triphenyltetrazolium chloride (TTC) (5) and $FeCl_3$ tests. The fact that **1** was positive to the TTC test and gave no fragment peak based on decarboxylation in the eims suggested that the acidity of this compound is mainly due not to a carboxylic but to a hydroxamic acid group. In the ^1H nmr spectrum of **1** in DMSO-d_6, four signals at δ 4.85 (1H, br d, J=6.0 Hz), 5.34, 11.74, and 16.98 (each 1H, br s) were assigned to the hydrogens in the OH groups of a secondary alcohol (ambiguous in the ^1H-nmr spectrum in $CDCl_3$), a secondary or tertiary alcohol, a hydroxamic acid, and a hydrogen-bonded phenol, respectively. From the ^1H- and ^{13}C-nmr spectral data of **1** in $CDCl_3$ and DMSO-d_6 with the aid of spin decoupling, ^1H-^1H COSY, INEPT or DEPT, ^{13}C-^1H COSY, and NOESY experiments, the presence of partial structures, **A** (1-substituted-2-methyldecalin-3-ene), **B** (a phenolic OH hydrogen-bonded with a car-

[1]Dedicated to the memory of Professor Edward Leete.

[2]An expected structure of this compound (tentatively named NFA) was reported at the 32nd Symposium on the Chemistry of Natural Products, Chiba, Japan, October 1990 [*Chem. Abstr.*, **115**, 49190u (1991)]. In this paper, the structure is partly revised on the basis of the nmr spectral data.

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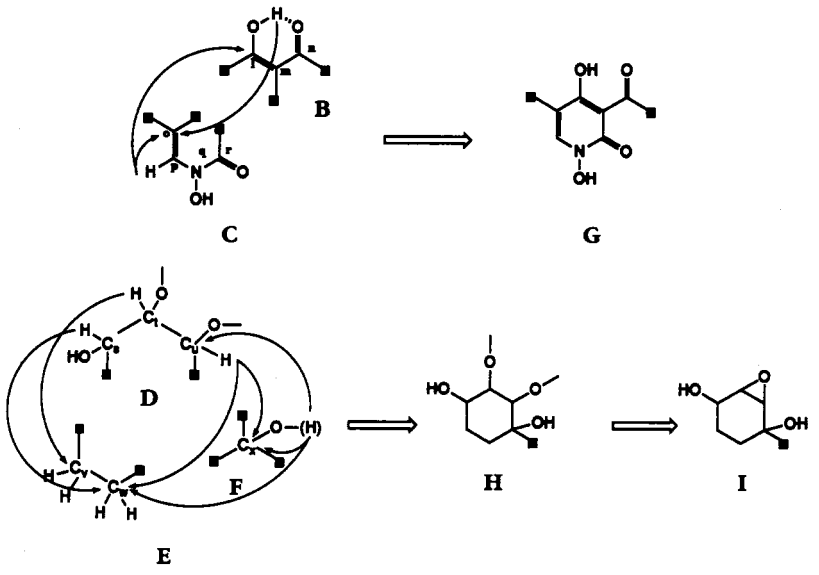
bonyl at the peri position), **C** [a hydroxamic acid with an olefinic proton at *a* position, $>C_o=C_pH\text{-}N_q(\text{-}OH)\text{-}C_r(=O)\text{-}$], **D** [$\text{-}C_sH(OH)\text{-}C_tH(O\text{-})\text{-}C_uH(O\text{-})\text{-}$], **E** ($\text{-}C_vH_2\text{-}C_wH_2\text{-}$), and **F** [$\text{-}C_x(O\text{-})<$] was suggested (see Table 1). The ^{1}H-detected multiple-bond heteronuclear multiple quantum coherence (HMBC) experiment of **1** in $CDCl_3$ and DMSO-d_6 suggested that together moieties **B** and **C** constructed a 1,4-dihydroxy-3-carbonyl-5-substituted-2(1*H*)-pyridone moiety **G**, because of the presence of significant cross peaks between C_pH in **C** and C_l in **B**, and between C_lOH in **B** and C_o in **C** (Scheme 1). Compound **1** afforded a dihydro derivative **2** on catalytic hydrogenation. The signals of two olefinic protons in **A** at δ 5.40 (1H, dt, $J_1=9.9, J_2=J_3=1.7$ Hz) and 5.59 (1H, ddd, $J_1=9.9, J_2=4.7, J_3=2.8$ Hz) in the ^{1}H nmr spectrum of **1** in $CDCl_3$ were replaced with the signals of two aliphatic methylenes at δ 1.33–1.40 (4H, m) in the ^{1}H-nmr spectrum of **2** (Tables 1 and 2). On methylation with CH_2N_2 in MeOH/Et_2O, **1** gave a dimethylether **3**. The ^{1}H-nmr spectrum of **3** in $CDCl_3$ showed that the hydroxyls of the phenolic and the hydroxamic acid in **G** were methylated during this reaction: δ 3.88, 4.07 (C-OMe, N-OMe). The dimethylether **3** was negative to the $FeCl_3$ test.

On acetylation with Ac_2O/pyridine, **1** gave a triacetate **4**, ^{1}H nmr ($CDCl_3$) δ 2.01,

TABLE 1. ^{13}C-nmr and ^{1}H-nmr Spectral Data for Partial Structures **A–F** in Fischerin [**1**].[a]

Partial Structure	^{13}C nmr Data	^{1}H nmr Data-1[b]	^{1}H nmr Data-2[c]
A	19.8 q, C_k	0.91 (3H) d (7.2)	2.52 m
	22.0 t, C_d	1.21 (2H) m	1.60 (2H) m
	26.1 t, C_e	1.21 m	1.60 (2H) m
		1.60 m	1.21 m
	29.0 t, C_c	1.39 (2H) m	2.24 dd (10.2, 3.9)
	30.2 t, C_f	1.21 m	1.60 m, 2.02 m
		1.60 m	1.21 m, 2.02 m
	37.0 d, C_g	2.02 m	2.24 dd (10.2, 3.9), 5.40 dt (9.9, 1.7), 5.59 ddd (9.9, 4.7, 2.8)
	37.6 d, C_i	2.52 m	4.68 t (10.2), 5.40 dt (9.9, 1.7), 5.59 ddd (9.9, 4.7, 2.8)
	38.1 d, C_b	2.24 dd (10.2, 3.9)	4.68 t (10.2)
	48.9 d, C_a	4.68 t (10.2)	2.24 dd (10.2, 3.9), 2.52 m
	131.2 d, C_j	5.40 dt (9.9, 1.7)	5.59 ddd (9.9, 4.7, 2.8)
	131.3 d, C_h	5.59 ddd (9.9, 4.7, 2.8)	5.40 dt (9.9, 1.7)
B	107.4 s, C_m		
	173.2 s, C_l	17.60 s[d], [16.98 br s][e], OH	
	215.6 s, C_n		
C	113.8 s, C_o		
	134.2 d, C_p	7.93 s	
	157.4 s, C_r		
	N_q	ca. 10.00 brs[f], [11.74 br s][e], OH	
D	56.2 d, C_t	3.50 t (3.9)	4.15 dd (8.0, 3.9)
	58.7 d, C_u	3.52 d (3.9)	
	63.8 d, C_s	4.15 dd (8.0, 3.9) [4.85 br d (6.0)][e], OH	
E	25.7 t, C_v	1.45 m	1.83 m, 1.92 m
		1.92 m	1.45 m, 1.83 m
	28.4 t, C_w	1.83(2H) m	
F	70.6 s, C_x		
■-(O)-H		4.15 s, [5.34 br s][e], OH	

[a] δ (ppm) from TMS in $CDCl_3$, (coupling constants in parentheses).
[b] Correlated with the ^{13}C-nmr data in ^{13}C-^{1}H COSY experiment.
[c] Correlated with the ^{1}H-nmr data-1 in spin decoupling and ^{1}H-^{1}H COSY experiments.
[d] Assigned by comparison of the ^{1}H-nmr spectrum of **1** with that of **3**.
[e] Observed in DMSO-d_6.
[f] Assigned by comparison of the ^{1}H-nmr spectrum of **1** with those of **3** and **4**.



SCHEME 1. Construction of the partial structures **G** from **B** and **C**, and **I** from **D**, **E**, and **F** via **H**.
→: HMBC data of **1** in $CDCl_3$ and DMSO-d_6.

2.04, 2.31 (each 3H, s), soluble in 1 N NaOH, insoluble in 5% $NaHCO_3$, positive to the $FeCl_3$ test, negative to the TTC test. Comparison of the ^{1}H-nmr spectrum of **4** with that of **1** in $CDCl_3$ indicated that the signals of the hydroxamic acid and the secondary or tertiary alcoholic OH disappeared, and the signal of C_sH in **D** was shifted to δ 5.17 (+1.02). Comparison of the ^{13}C-nmr spectrum of **4** with that of **1** showed that the signals of C_s–C_x, respectively in **D**, **E**, and **F** were observed at δ 67.7 (+3.9), 54.0 (−2.2), 56.0 (−2.7), 21.9 (−3.8), 27.5 (−0.9), and 77.2 (+6.6) in $CDCl_3$, and δ 70.3 (+2.6), 55.8 (−2.6), 58.6 (−2.5), 22.7 (−3.7), 28.5 (−3.8), and 78.2 (+7.0) in CD_3OD (Table 3). These results indicated that the hydroxyls at C_s and C_x were acetylated to afford **4**, suggesting that the hydroxyl group in **F** should be a tertiary, which was assignable to the signal at δ 5.34 in the ^{1}H-nmr spectrum of **1** in DMSO-d_6. The signals of C_sH, C_tH, C_uH, C_vH_2, and C_wH_2, respectively, were observed at δ 4.15 (1H, dd, J_1=8.0, J_2=3.9 Hz), 3.50 (1H, t, J=3.9 Hz), 3.52 (1H, d, J=3.9 Hz), 1.45, 1.92 (each 1H, m), and 1.83 (2H, m) in the ^{1}H-nmr spectrum of **1** in $CDCl_3$, and δ 5.17 (1H, td, J_1=5.9, J_2=J_3=3.5 Hz), 3.52 (1H, t, J=3.5 Hz), 3.96 (1H, d, J=3.5 Hz), 1.42, 1.89 (each 1H, m), and 2.15 (2H, m) in the spectrum of **4** in $CDCl_3$, respectively (Table 2). Comparison of the ^{13}C nmr spectral data of C_s–C_x in **4** with the corresponding data in **1** on the basis of the acetylation shift rule (6,7) and the coupling pattern of the ^{1}H-nmr signals of C_sH–C_wH_2 of **4** with that of **1** suggested that C_sH, C_uH, and C_wH_2 were connected with C_vH_2, C_x, and C_x, respectively. Therefore, it was supposed that **D**, **E**, and **F** construct 1,4-dihydroxy-2,3-di-*O*-substituted-1-substituted-cyclohexane **H**. The presence of partial structure **H** was also supported by the fact that the cross peaks of C_sH/C_w, C_tH/C_v, C_uH/C_w, and C_uH/C_x in the HMBC spectra of **1** in $CDCl_3$ and DMSO-d_6 and **4** in CD_3OD, and also the cross peaks of C_xOH/C_x, C_xOH/C_u, and C_xOH/C_w in the HMBC spectrum of **1** in DMSO-d_6, were observed. As the molecular formula of **1** has only four hydroxyls, **H** was assumed to be 1,4-dihydroxy-2,3-epoxy-1-substituted-cyclohexane **I** (Scheme 1). The presence of the **A**, **G**, and **I** moieties in **1** was also supported by the data from the 2D INADEQUATE spectrum of **1** in CD_3OD (see Experimental). Thus, the structure of fischerin was

TABLE 2. ^{1}H-nmr Data for Fischerin [**1**], Dihydrofischerin [**2**], Fischerin Dimethylether [**3**], and Fischerin Triacetate [**4**].[a]

Proton	Compound						
	1			**2**	**3**	**4**	
	in $CDCl_3$[b]	in DMSO-d_6[b]	in CD_3OD[c]	in $CDCl_3$[b]	in $CDCl_3$[c]	in $CDCl_3$[b]	in CD_3OD[b]
1-OH	ca. 10.0 br s	11.74 br.s					
4-OH	17.60 s	16.98 br.s		17.74 s		17.76 s	
H-6	7.93 s	7.94 s	8.02 s	8.00 s	7.69 s	7.54 s	8.15 s
H-8	4.68 t (10.2)	4.93 t (10.3)	4.95 dd (11.0,9.9)	4.78 t (11.1)	3.42 t (4.0)	4.59 dd (9.7,9.5)	4.75 t (10.5)
H-9	2.24 dd (10.2,3.9)	2.17 m	2.26 m	2.03 dd (10.3,4.7)	2.26 m	2.19 m	2.25 m
H-10	1.39 (2H) m	1.20 m	1.43 m	1.51 (2H) m	1.58 (2H) m	1.42 (2H) m	1.45 (2H) m
		1.66 m	1.47 m				
H-11	1.21 (2H) m	1.29 m	1.30 (2H) m	1.33~1.40 (2H) m	1.37 (2H) m	1.27 (2H) m	1.33 (2H) m
		1.37 m					
H-12	1.21 m	1.20 m	1.25 m	1.33~1.40 m	1.26 m	1.19 m	1.20 m
	1.60 m	1.60 m	1.65 m	1.68 m	1.58 m	1.57 m	1.67 m
H-13	1.21 m	1.37 (2H) m	1.33 m	1.33~1.40 m	1.26 m	1.27 m	1.20 m
	1.60 m		1.68 m	1.66 m	1.64 m	1.57 m	1.67 m
H-14	2.02 m	2.05 br.d (3.3)	2.06 br.d (6.6)	1.99 dd (9.6,4.7)	2.26 m	2.00 m	2.02 br.d (5.2)
H-15	5.59 ddd (9.9,4.7,2.8)	5.64 ddd (9.6,4.7,2.6)	5.64 ddd (9.9,5.0,2.8)	1.33~1.40 (2H) m	5.53 dt (10.1,2.4)	5.55 ddd (9.9,4.5,2.7)	5.64 ddd (9.9,4.8,2.5)
H-16	5.40 dt (9.9,1.7)	5.45 br.d (10.3)	5.45 dt (9.9,1.7)	1.33~1.40 (2H) m	5.48 br.d (10.1)	5.38 br.d (9.9)	5.44 dt (9.9,1.5)
H-17	2.52 m	2.48 m	2.57 tt (7.1,3.3)	2.20 d (11.1)	2.64 m	2.49 m	2.55 tt (7.6,2.3)
18-Me	0.91 (3H) d (7.2)	0.92 (3H) d (6.8)	0.98 (3H) d (7.1)	0.89 (3H) d (6.4)	1.19 (3H) d (7.3)	0.92 (3H) d (7.3)	0.96 (3H) d (7.3)
19-OH	4.15 s	5.34 br.s			3.58 br s.		
H-20	3.52 d (3.9)	3.56 d (3.9)	3.66 dd (3.9,0.6)	3.57 d (3.4)	3.45 d (3.7)	3.96 d (3.5)	4.05 d (4.0)
H-21	3.50 t (3.9)	3.24 t (3.0)	3.41 dd (3.7,2.9)	3.58 t (3.4)	3.55 t (3.7)	3.52 t (3.5)	3.57 dd (3.6,2.8)
H-22	4.15 dd (8.0,3.9)	3.94 m	4.12 td (6.4,2.8)	4.22 br.d (4.3)	4.21 br.d (3.0)	5.17 td (5.9,3.5)	5.22 m
22-OH		4.85 br.d (6.0)					
H-23	1.92 m	1.60 m	1.82 m	1.57 m	1.47 ddd (11.9,5.5,2.6)	1.42 m	1.45 m
	1.45 m	1.08 m	1.37 m	1.90 m	1.97 tdd (12.5,5.2,2.9)	1.89 m	1.90 m
H-24	1.83 (2H) m	1.45 m	1.70 m	1.75 m	1.74 ddd (11.3,7.3,2.7)	2.15 (2H) m	2.12 m
		2.22 m	2.23 m	1.88 m	1.89 td (12.7,2.3)		2.44 m
OCH_3					3.88 (3H) s		
					4.07 (3H) s		
$OCOCH_3$						2.01 (3H) s	2.04 (3H) s
						2.04 (3H) s	2.06 (3H) s
						2.31 (3H) s	2.37 (3H) s

[a]δ (ppm) from TMS, coupling constants in parentheses.
[b]Recorded at 500 MHz.
[c]Recorded at 400 MHz.

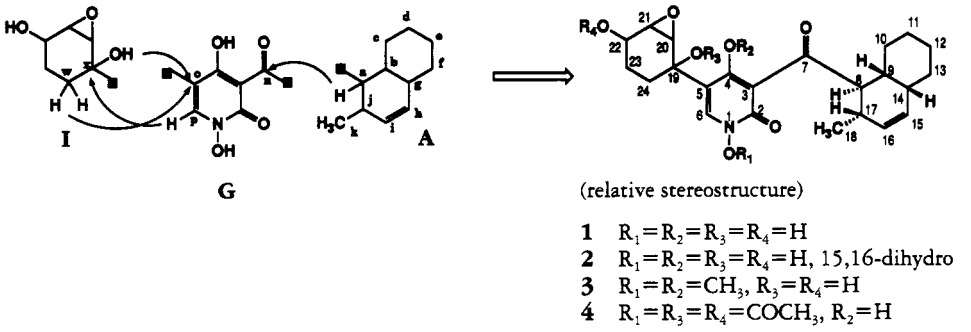
TABLE 3. ^{13}C nmr Data for Fischerin [**1**], Fischerin Dimethylether [**3**], and Fischerin Triacetate [**4**].[a]

Carbon	Compound					
	1			**3**	**4**	
	in $CDCl_3$[b]	in DMSO-d_6[c]	in CD_3OD[d]	in $CDCl_3$[d]	in $CDCl_3$[c]	in CD_3OD[c]
C-2	157.4 s	157.9 s	160.9 s	157.0 s	156.4 s	158.6 s
C-3	107.4 s	108.0 s	110.3 s	117.3 s	108.8 s	109.9 s
C-4	173.2 s	174.3 s	176.1 s	165.2 s	176.1 s	176.6 s
C-5	113.8 s	112.5 s	115.3 s	117.3 s	111.0 s	112.2 s
C-6	134.2 d	139.7 d	141.1 d	133.7 d	139.4 d	142.5 d
C-7	215.6 s	213.9 s	216.9 s	204.8 s	214.9 s	216.2 s
C-8	48.9 d	47.0 d	50.0 d	56.2 d	48.5 d	50.0 d
C-9	38.1 d	37.7 d	40.4 d	34.7 d	38.0 d	39.7 d
C-10	29.0 t	29.5 t	30.7 t	29.4 t	29.0 t	30.0 t
C-11	22.0 t	21.0 t	23.4 t	23.3 t	22.0 t	22.6 t
C-12	26.1 t	25.7 t	28.1 t	25.5 t	26.0 t	27.4 t
C-13	30.2 t	28.3 t	32.0 t	31.1 t	30.2 t	31.3 t
C-14	37.0 d	36.7 d	39.4 d	31.6 d	36.9 d	38.8 d
C-15	131.3 d	131.1 d	133.1 d	131.0 d	131.3 d	132.4 d
C-16	131.2 d	131.3 d	133.0 d	130.3 d	131.0 d	132.3 d
C-17	37.6 d	37.1 d	39.6 d	33.0 d	37.1 d	38.9 d
C-18	19.8 q	19.3 q	20.8 q	22.3 q	19.9 q	20.1 q
C-19	70.6 s	68.1 s	71.2 s	70.7 s	77.0 s	78.2 s
C-20	58.7 d	59.9 d	61.1 d	59.3 d	56.0 d	58.6 d
C-21	56.2 d	56.8 d	58.4 d	56.2 d	54.0 d	55.8 d
C-22	63.8 d	65.8 d	67.7 d	64.0 d	67.7 d	70.3 d
C-23	25.7 t	24.6 t	26.4 t	25.8 t	21.9 t	22.7 t
C-24	28.4 t	31.8 t	32.3 t	28.5 t	27.5 t	28.5 t
OCH_3				65.0 q		
				63.0 q		
$OCOCH_3$					17.9 q	17.7 q
					21.0 q	20.8 q
					21.5 q	21.5 q
$OCOCH_3$					166.4 s	168.3 s
					169.9 s	171.5 s
					170.6 s	172.3 s

[a]δ (ppm) from TMS.
[b]Recorded at 67.8 MHz.
[c]Recorded at 125.65 MHz.
[d]Recorded at 100.40 MHz.

constructed with **A**, **G**, and **I** to be **1** as shown in Scheme 2, because **1** gave the significant cross peaks of C_aH/C_n and C_pH/C_x in $CDCl_3$ and C_aH/C_n, C_pH/C_x, C_wH_2/C_o, and C_xOH/C_o in DMSO-d_6, and **4** gave those of C_aH/C_n, C_pH/C_x, and C_wH_2/C_o in CD_3OD in the HMBC experiment. It is well known that the signals of the carbons at the part of ring juncture in a *cis*-decalin appear at δ 36.5–37.0, and those in a *trans*-decalin appear at δ 43.4–44.2 in the ^{13}C-nmr spectrum (8). The signals of C-9 and C-14 were observed at δ 38.1 and 37.0 in the ^{13}C-nmr spectrum of **1** in $CDCl_3$, suggesting that the ring juncture between C-9 and C-14 in **1** is cis. The coupling constants between H-8 and H-9, and between H-8 and H-17, were observed to be 10.2 Hz in the ^1H nmr spectrum of **1** in $CDCl_3$ {H-8 δ 4.68 (t, J=10.2 Hz), see Table 2}, suggesting that the configurations between H-8 and H-9, and between H-8 and H-17, are trans diaxial. This assumption was also supported by the fact that a significant nOe was observed between H-9 and H-17 in the NOESY spectrum of **1** in $CDCl_3$. Accordingly, the structure of fischerin, including relative stereostructure, was deduced to be **1** as shown in Scheme 2.

As fungal metabolites which include 1,4-dihydroxy-2(1*H*)-pyridone or its homologous moieties, several compounds, namely tenellin [**5**], bassianin [**6**] from *Beauveria tenella* and *Beauveria bassiana* (9–11), ilicicolin H [**7**] from *Cylindrocladium ilicicola* (12), funiculosin [**8**] from *Penicillium funiculosum* (13), harzianopyridone [**9**] from *Trichoderma harzianum* (14), and leporin A [**10**] from *Aspergillus leporis* (15), have so far been isolated



Scheme 2. Structure construction of fischerin [**1**] from the partial structures **A**, **G**, and **I**. →: HMBC data of **1** in DMSO-d_6.

(Figure 1). They have been isolated as fungal pigments (**5**, **6**), antibiotics (**7**, **8**, **9**), and an anti-insectan (**10**). Comparison of the ^{13}C-nmr spectral data of **1** with those of **5** and **7** described in the literature indicated that the spectral data at positions 1–7 of **1** are very similar to the corresponding data of **5** and **7**. The biosynthesis of these metabolites through a pathway that starts from polyketide and phenylalanine precursors and proceeds to **5** or **7** via an intermediate formed from condensation of the two precursors was already reported (9,10,16). Compound **1** may also be biosynthesized through a pathway similar to that for biosynthesis of **5** or **7** because of the structural similarity of **1** to **5** or **7**, as shown in Scheme 3.

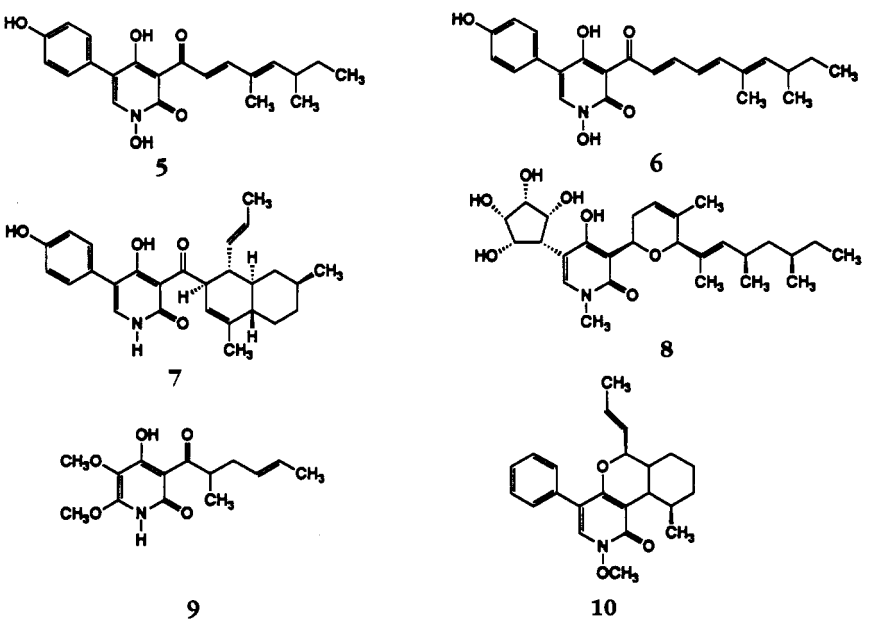
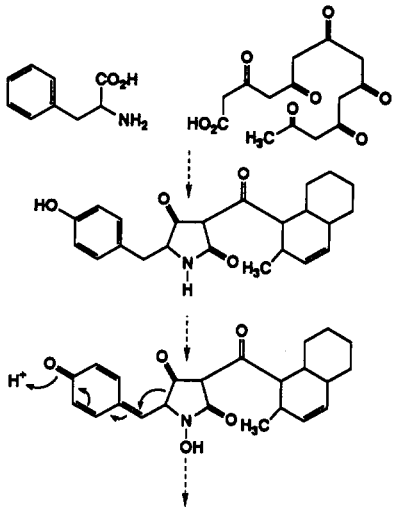


FIGURE 1. Structures of the fungal metabolites including 1,4-dihydroxy-2(1*H*)-pyridone or its homologous moieties, **5**, **6** (9,10,11), **7** (12), **8** (13), **9** (14), and **10** (15).



1

SCHEME 3. Biogenesis of **1**.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—The mp's were measured on a Yanagimoto micro mp apparatus (hot stage type) and are uncorrected. The optical rotations were measured with a JASCO DIP-140 digital polarimeter. The ir spectra were recorded with a Hitachi IR 260-10 spectrometer, the uv spectra with a Hitachi U-3400 spectrometer, the eims spectra with a Hitachi M-60, the hreims spectra with a Hitachi RMU-7M, the fabms with a JEOL JMS-SX102, the ^{1}H-nmr spectra with a JEOL JNM-GSX270, -GSX400, -GSX500, -A500 or a Varian XL-VXR300 spectrometer at 270, 400, 500, 500 or 300 MHz, the ^{13}C-nmr spectra with a JEOL JNM-GSX270, -GSX400, -GSX500, -A500 or a Varian XL-VXR300 spectrometer at 67.8, 100.4, 125.65, 125.65, or 75 MHz. Chemical shifts are expressed in δ (ppm) values from TMS as an internal standard. The tlc analyses were carried out with Si gel (Merck 60F254) plates or reversed-phase Si gel (Merck RP-18 F254S) plates, the cc separations with Si gel columns (Wakogel C-200 or Nacalai-Tesque Si gel 60), the flash cc separations with Si gel columns with a handy pump (NRK UP2), the mplc separations with an mplc system (column Kusano prepacked ODS 22 i.d.×100 mm, pump Senshu SSC-3100, controller Senshu SSC-3110, uv detector Senshu SSC Y1000 or Y3000). The toxicity of each sample was examined by ip injection of 0.1 ml of a solution of each sample in DMSO into each mouse (ddY, male, 20–25 g) and by observation of the behavior of the injected mice within 72 h (3 mice were employed for each sample).

ISOLATION OF FISCHERIN [**1**].—*N. fischeri* var. *fischeri* CBM-FA-0156 (isolation strain 79-SH-413-1) (A voucher specimen is deposited at the Natural History Museum and Institute, Chiba, Japan.) (1,2) was cultured on rice medium (17) (200 g/flask×100) at 25° for 28 days. The cultures were extracted with Me_2CO (300 ml/flask×100) by stirring at room temperature for 12 h 3 times to afford an Me_2CO extract (122 g) after evaporation of the solvent. The Me_2CO extract was treated with *n*-hexane (200 ml) 2 times to remove the fatty portion. The defatted extract (73 g) was then treated with $CHCl_3$ (200 ml) 3 times to obtain a $CHCl_3$-soluble portion (56 g). The $CHCl_3$-soluble portion was chromatographed on a Si gel column with C_6H_6, $CHCl_3$, $CHCl_3$-Me_2CO (1:1), and Me_2CO, successively. The fraction eluted with C_6H_6 and $CHCl_3$ gave a non-polar fraction which caused tremors in mice. The fraction eluted with $CHCl_3$-Me_2CO (1:1) gave a polar fraction (16.6 g) which caused lethal peritonitis in mice at a dose of 500 mg/kg. The polar fraction was separated chromatographically on a Sephadex LH-20 column with $CHCl_3$-MeOH (1:1), on a flash chromatographic column of Si gel impregnated with 6% (w/w) H_2O with $CHCl_3$-MeOH (30:1), and on an mplc column of ODS with MeOH-H_2O (65:35) at a flow rate of 8.0 ml/min, successively, to afford **1** (709 mg), which gave single spots on both tlc plates of Si gel and reversed-phase Si gel developed with $CHCl_3$-MeOH (9:1) and MeOH-H_2O (3:1), respectively.

Compound **1**.—Pale yellow amorphous powder: $[\alpha]^{26}D$ −65.0° (*c*=0.52, $CHCl_3$); hreims *m/z* $[M]^+$ 431.1942 (calcd for $C_{23}H_{29}NO_7$, 431.1944); eims *m/z* (%) $[M]^+$ 431 (41); fabms *m/z* $[M+H]^+$ 432, +KI *m/z* $[M+K]^+$ 470; ir ($CHCl_3$) 3550 (OH), 3200 (OH), 2920, 2840, 1640 (C=O), 1600, 1540, 1450, 1440

cm^{-1}; uv max (MeOH) 213 (ε 13000), 233 (7700), 290 (4800), 343 (3800) nm; ^1H nmr see Table 2; ^{13}C nmr see Table 3. The 2D INADEQUATE spectrum of **1** (230 mg) in CD_3OD (0.75 ml) indicated the presence of the C-C and/or C=C bond chains (a) and (b) at a condition of pulse repetition time=5.027 sec (acquisition time=0.027 sec), τ=6.25 msec ($J_{c\text{-}c}$=40 Hz), and (c) at that of pulse repetition time=5.134 sec (acquisition time=0.134 sec), τ=4.17 msec ($J_{c\text{-}c}$=60 Hz), as follows: (a) C_kH_3-C_jH(-C_iH=)-C_hH(−)-C_bH(-C_cH_2-C_dH_2-)-C_gH(-C_hH=)-C_fH_2-C_eH_2- (b) -C_rH_2-C_sH(-OH)-C_tH(-O)-C_uH(-O)-C_x(-O) (−)-C_wH_2-, (c) -C_pH=C_o (−)-C_l (-OH)=C_m (−)-C_n(= O)-C_b=C_i-.

CATALYTIC HYDROGENATION OF FISCHERIN [**1**].—A suspension of 5% Pd/C (5.5 mg) in EtOH (1.0 ml) was stirred vigorously under H_2 gas at room temperature for 15 min. A solution of **1** (5.0 mg) in EtOH (1.5 ml) was added to the suspension. The reaction mixture was stirred under H_2 gas at room temperature for 2 h. After removal of the catalyst, the solvent was evaporated to give a residual product, which was purified through a small ODS column (Waters, Sep-Pak C18) with MeOH-H_2O (7:3) to afford **2** (4.2 mg): pale yellow amorphous powder; ir ($CHCl_3$) 3550, 3200, 2920, 2850, 1640, 1600, 1550, 1440 cm^{-1}; ^1H nmr see Table 2.

METHYLATION OF FISCHERIN [**1**].—A solution of CH_2N_2 in Et_2O (1.0 ml) was added to a solution of **1** (30 mg) in MeOH (0.3 ml). The reaction mixture was stirred at room temperature overnight. Evaporation of the solvent gave a product mixture, which was separated chromatographically on a Si gel column with $CHCl_3$-MeOH (20:1), followed by preparative tlc using a Si gel plate with $CHCl_3$-MeOH (10:1) to afford **3** (7.3 mg): colorless amorphous powder; ir ($CHCl_3$) 3560, 2920, 2850, 1640, 1600 cm^{-1}; ^1H nmr see Table 2; ^{13}C nmr see Table 3.

ACETYLATION OF FISCHERIN [**1**].—A solution of **1** (85 mg) in Ac_2O (1.0 ml) and pyridine (1.0 ml) was maintained at room temperature for 19 h. After addition of ice-H_2O to the solution, the mixture was extracted with EtOAc. After evaporation of the solvent, a solid residue was purified on a flash chromatographic column of Si gel with $CHCl_3$-Me_2CO (40:1), followed by recrystallization from *n*-hexane and Me_2CO, successively, to afford **4** (38 mg): colorless needles; mp 121–123°; $[\alpha]^{22}D$ −46.0° (*c*=0.44, $CHCl_3$); fabms *m/z* $[M+H]^+$ 558, +KI *m/z* $[M+K]^+$ 596; ir ($CHCl_3$) 3350, 2920, 2850, 1820, 1735, 1675, 1600 cm^{-1}; uv max (MeOH) 231 (ε 9500), 282 (4900), 332 (8500) nm; ^1H nmr see Table 2; ^{13}C nmr see Table 3.

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