STRUCTURES OF FIBROSTATINS, NEW INHIBITORS OF PROLYL HYDROXYLASE

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The structures of six inhibitors of prolyl hydroxylase, fibrostatins A, B, C, D, E and F produced by a strain of *Streptomyces*, were deduced to be **1**, **2**, **3**, **4**, **5** and **6**, respectively, from chemical and spectroscopic evidence, especially from extensive ^{13}C NMR studies including selective decoupling and low power selective decoupling experiments monitored by ^{13}C-^{1}H long-range couplings. These compounds are the first naturally occurring 2,6,7- or 3,6,7-tri-substituted or 2,3,6,7-tetra-substituted 5-hydroxy-1,4-naphthoquinone inhibitors possessing *N*-acetyl-L-cystein-*S*-yl moieties in the molecule.

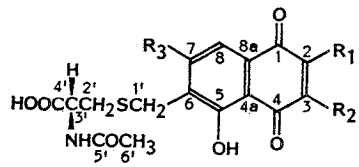
During the course of a screening program directed toward the isolation and evaluation of prolyl hydroxylase inhibitors of microbial origin, six new inhibitors were found in the culture broth of *Streptomyces catenulae* subsp. *griseospora* No. 23924.

In a previous paper[1], we reported on taxonomy, fermentation, isolation, physico-chemical and biological properties of these inhibitors. In this paper, the structural determination of six new inhibitors, fibrostatins A (**1**), B (**2**), C (**3**), D (**4**), E (**5**) and F (**6**) are reported.

Fibrostatin B (**2**) was selected as a standard for the structural determination of other fibrostatins and it was obtained as yellowish orange crystals, mp 200~202°C, $[\alpha]_D^{23}$ −90° (*c* 0.51, MeOH). The electron impact mass spectrum (EI-MS) showed the molecular ion at *m/z* 423. The ^{13}C NMR spectrum revealed the presence of nineteen carbons; seven aliphatic carbons ($CH_3\times2$, $CH_2\times2$, $=CH\times1$, $CH_3O\times2$), eight aromatic carbons ($CH\times1$, $C\times4$, $CO\times3$) and four carbonyl carbons (Table 3). The molecular formula of **2** was determined to be $C_{19}H_{21}NO_8S$ by elemental analysis, high-resolution MS and carbon number in the ^{13}C NMR. Compound **2** gave a positive response with ferric chloride, methanolic magnesium acetate and Rydon-Smith reagents, while ninhydrin and Ehrlich tests were negative. The IR spectrum of **2** disclosed absorption bands due to hydroxyl, chelated carbonyl, non-chelated carbonyl, carboxyl and amide groups. The UV spectrum of **2** in methanol, having absorption maxima at 220, 262 (sh), 268, 308 and 420 nm, indicated that it was most likely a derivative of 5-hydroxy-1,4-naphthoquinone[2]. The presence of a quinone group was supported by the signals at δ 179.2 (s, C-1) and 189.5 (s, C-4) in the ^{13}C NMR spectrum, the latter carbonyl is hydrogen-bonded to the phenolic hydroxyl group[3]. The ^{1}H NMR spectrum of **2** showed the presence of two methyl groups, two methylene groups, a methine group, two methoxyl groups, an aromatic proton, an imino group and a chelated hydroxyl group (Table 2).

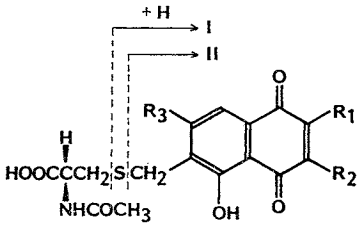
On catalytic hydrogenation with Raney nickel as a catalyst, **2** liberated *N*-acetyl-L-alanine (**2a**)[4~6] and a chromophoric substance **2b** (Fig. 2). The identity of **2a** was confirmed by direct comparison of its mp, $[\alpha]_D$, TLC, IR, ^{1}H NMR and EI-MS with those of an authentic sample of *N*-acetyl-L-alanine.

Fig. 1. Structures of fibrostatins.



Fibrostatin A (1)	R_1=H	R_2=CH_3	R_3=OCH_3
Fibrostatin B (2)	R_1=OCH_3	R_2=CH_3	R_3=OCH_3
Fibrostatin C (3)	R_1=OCH_3	R_2=H	R_3=OCH_3
Fibrostatin D (4)	R_1=OCH_3	R_2=CH_3	R_3=OH
Fibrostatin E (5)	R_1=H	R_2=CH_2OH	R_3=OCH_3
Fibrostatin F (6)	R_1=OCH_3	R_2=CH_2OH	R_3=OCH_3

Table 1. Diagnostic mass fragmentation for fibrostatins.



	1 (R_1=H, R_2=CH_3, R_3=OCH_3)	2 (R_1, R_3=OCH_3, R_2=CH_3)	3 (R_1, R_3=OCH_3, R_2=H)
	393 (M^+)	423 (M^+)	409 (M^+)
I	264	294	280
II	231	261	247

	4 (R_1=OCH_3, R_2=CH_3, R_3=OH)	5 (R_1=H, R_2=CH_2OH, R_3=OCH_3)	6 (R_1, R_3=OCH_3, R_2=CH_2OH)
	409 (M^+)	410 $(M+1)^+$*	439 (M^+)
I	280	—	—
II	247	247*	277

* Determined by FAB-MS.
—: Not determined.

The molecular formula of **2b** was determined to be $C_{14}H_{14}O_5$ (*m/z* 262 (M^+)) by the elemental analysis and EI-MS. Examination of the UV and ^1H NMR spectra of **2b** suggested that it was a 5-hydroxy-1,4-naphthoquinone derivative having two methyl and two methoxyl groups.

In order to confirm the structure of **2b**, an X-ray structural analysis was carried out. Single crystals of **2b** were grown from an hexane - EtOAc solution by slow evaporation. The molecular structure of **2b** was determined to be 5-hydroxy-2,7-dimethoxy-3,6-dimethyl-1,4-naphthoquinone as shown in Fig. 3.

The ^1H NMR signals of **2a** and **2b** in DMSO-d_6 showed similar patterns to the corresponding signals in **2** (Table 2 and Experimental). However, methyl signals at δ 1.24 (d, J=7.0 Hz) in the spectrum of **2a** and δ 2.05 (s) in that of **2b** were not observed in the spectrum of **2** in DMSO-d_6. Conversely, two methylene signals at δ 2.70 (dd, J=8.8 and 13.7 Hz), 2.89 (dd, J=4.9 and 13.7 Hz), 3.65 (d, J=13.1 Hz) and 3.77 (d, J=13.1 Hz) were observed in the spectrum of **2** and absent in those of

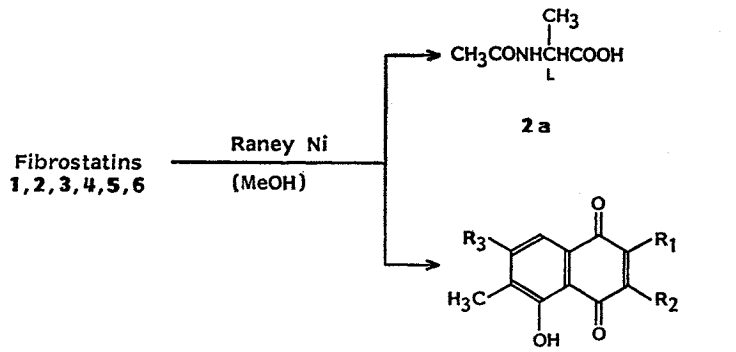
Table 2. ^{1}H NMR data for fibrostatins in DMSO-d_6 (400 MHz) (δ in ppm, J in Hz, internal reference TMS).

	1			2		
R_1	6.89	q	J=1.8	4.03	s	
R_2	2.09	d	J=1.8	1.95	s	
5-OH	12.40	s		12.59	s	
R_3	3.96	s		3.95	s	
8-H	7.09	s		7.11	s	
1′-CH_2	3.66 and	d	J=12.9	3.65 and	d	J=13.1
	3.78	d	J=12.9	3.77	d	J=13.1
2′-CH_2	2.71 and	dd	J=8.8, 13.6	2.70 and	dd	J=8.8, 13.7
	2.91	dd	J=5.0, 13.6	2.89	dd	J=4.9, 13.7
3′-H	4.47	dt like	J=5.0, 8.3, 8.8	4.47	dt like	J=4.9, 8.1, 8.8
CONH	8.18	d	J=8.3	8.18	d	J=8.1
6′-CH_3	1.86	s		1.86	s	
	3			**4**		
R_1	3.88	s		4.00	s	
R_2	6.26	s		1.94	s	
5-OH	12.73	s		12.79	s	
R_3	3.95	s		—	—	
8-H	7.15	s		7.03	s	
1′-CH_2	3.66 and	d	J=12.9	3.64 and	d	J=12.8
	3.77	d	J=12.9	3.76	d	J=12.8
2′-CH_2	2.71 and	dd	J=8.8, 13.7	2.73 and	dd	J=8.8, 13.5
	2.91	dd	J=4.9, 13.7	2.92	dd	J=4.8, 13.5
3′-H	4.47	dt like	J=4.9, 8.1, 8.8	4.48	dt like	J=4.8, 8.1, 8.8
CONH	8.18	d	J=8.1	8.17	d	J=8.1
6′-CH_3	1.85	s		1.85	s	
	5			**6**		
R_1	6.82	t	J=2.2	4.10	s	
R_2	4.47 (CH_2)	br d	J=2.2	4.36 (CH_2)	d	J=3.7
	5.47 (OH)	br s		4.91 (OH)	br t	
5-OH	12.29	s		12.69	s	
R_3	3.98	s		3.97	s	
8-H	7.15	s		7.15	s	
1′-CH_2	3.67 and	d	J=13.1	3.67 and	d	J=13.1
	3.79	d	J=13.1	3.80	d	J=13.1
2′-CH_2	2.71 and	dd	J=8.5, 13.7	2.70 and	dd	J=8.8, 13.7
	2.90	dd	J=4.9, 13.7	2.90	dd	J=4.9, 13.7
3′-H	4.47	dt like	J=4.9, 8.1, 8.5	4.47	dt like	J=4.9, 8.3, 8.8
CONH	8.18	d	J=8.1	8.20	d	J=8.3
6′-CH_3	1.85	s		1.86	s	

—: Not determined.

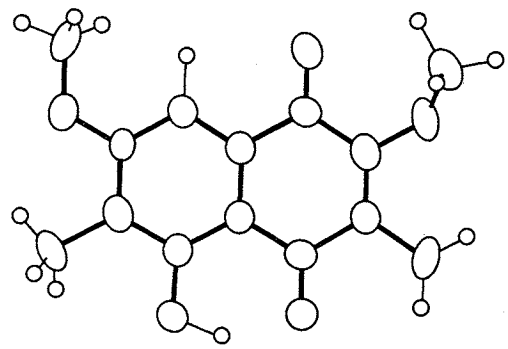
the degradation products. Taking into account the reaction mechanism for desulfurization, it was speculated that **2a** was derived from an *N*-acetyl-L-cystein-*S*-yl moiety in **2**. The occurrence of an *N*-acetyl-L-cystein-*S*-yl moiety in **2** was supported by the following spectroscopic data, *i.e.*, the signals at δ 1.86 (3H, s, $COCH_3$), 2.70 (1H, dd, J=8.8 and 13.7 Hz, *H*CH), 2.89 (1H, dd, J=4.9 and 13.7 Hz, HC*H*), 4.47 (1H, dt like, J=4.9, 8.1 and 8.8 Hz, CH) and 8.18 (1H, d, J=8.1 Hz, disappeared on D_2O exchange, NH) in the ^{1}H NMR spectrum (Table 2) combined with spin-decoupling, together with five carbon signals at δ 22.5 (q), 33.5 (t), 51.9 (d), 169.2 (s) and 172.2 (s) in the ^{13}C NMR spectrum (Table 3) determined by the off-resonance decoupling technique. These findings showed that the *N*-acetyl-L-cystein-*S*-yl moiety is bound to the methylene group at the 6-position of **2b**.

Fig. 2. Chemical degradation of fibrostatins.



1b, 5b	R_1=H	R_2=CH_3	R_3=OCH_3
2b, 6b	R_1=OCH_3	R_2=CH_3	R_3=OCH_3
3b	R_1=OCH_3	R_2=H	R_3=OCH_3
4b	R_1=OCH_3	R_2=CH_3	R_3=OH

Fig. 3. Molecular structure of **2b**.



In order to determine the structure of **2**, extensive ^{13}C NMR studies including selective decoupling experiments and low power selective decoupling (LPSD) experiments monitored by ^{13}C-^{1}H long-range couplings were conducted as shown in Table 4. In the ^{13}C NMR spectrum of **2** measured after addition of D_2O, when the signal of the aromatic proton at δ 7.11 (8-H) was irradiated by the LPSD method, the signals at δ 179.2 (d, ^{3}J=4.0 Hz, C-1), 108.4 (d, ^{3}J=5.4 and ^{3}J=6.0 Hz, C-4a) and 130.9 (d, ^{2}J=2.7 Hz, C-8a) were changed into singlets, and the signals at δ 120.3 (q, ^{2}J=6.0 and ^{3}J=6.0 Hz, C-6) and 161.7 (m, ^{2}J=1.3 and ^{3}J=3.7 Hz, C-7) were changed into a triplet and a sextet, respectively. On the other hand, irradiation of the center of the isolated methylene protons at δ 3.65 and 3.77 converted the signals at δ 159.1 (t, ^{3}J=4.0 Hz, C-5), 120.3 (q, ^{2}J=6.0 and ^{3}J=6.0 Hz, C-6), 161.7 (m, ^{2}J=1.3 and ^{3}J=3.7 Hz, C-7) and 33.5 (tq, ^{1}J=141, ^{2}J=4.7 and ^{3}J=4.7 Hz, C-2′) to a singlet, a doublet, a broad singlet and a broad triplet, respectively (Table 4, (c)). Furthermore, the signals due to C-4a, C-5 and C-6 were also altered to a doublet, a triplet and a quartet, respectively, upon D_2O treatment, which demonstrated the presence of the phenolic hydroxyl group at the 5-position of **2** (Table 4, (a)). The carbon assignment corresponding to the *N*-acetyl-L-cystein-*S*-yl moiety was also confirmed in a similar manner. These data were also supported by the ^{1}H and ^{13}C NMR data (Tables 2 and 3), and by the examination of the diagnostic mass fragmentation, which gave strong peaks at *m/z* 294 and 261 due to the fragment ions I and II, respectively (Table 1). From these observations, the structure of fibrostatin B was formulated as **2** and this provided a reference for the determination of the structures of the other fibrostatins.

Fibrostatin A (**1**) was obtained as orange-yellow crystals, mp 186~188°C, $[\alpha]_D^{23}$ −91° (*c* 0.5, MeOH). The EI-MS showed the molecular ion peak at *m/z* 393. The molecular formula of **1** was determined to be $C_{18}H_{19}NO_7S$ by elemental analysis, high-resolution MS and carbon number in the

Table 3. ^{13}C NMR data for fibrostatins in DMSO-d_6 (100 MHz) (δ in ppm, internal reference TMS)[a].

Carbon No.	1	2	3	Carbon No.	4	5	6
1	183.2 s	179.2 s	178.2 s	1	179.6 s	183.2 s	180.3 s
2	135.2 d	157.5 s	160.5 s	2	157.4 s	131.9 d	158.5 s
R_1	—	60.8 q	56.9 q	R_1	60.8 q	—	61.6 q
3	147.9 s	129.6 s	109.0 d	3	130.2 s	151.5 s	130.5 s
R_2	15.4 q	8.5 q	—	R_2	8.6 q	57.2 t	51.2 t
4	188.8 s	189.5 s	189.7 s	4	189.1 s	188.4 s	189.3 s
4a	109.4 s	108.4 s	108.3 s	4a	107.1 s	109.6 s	108.5 s
5	159.6 s	159.1 s	159.2 s	5	160.5 s	159.7 s	159.3 s
6	119.9 s	120.3 s	121.0 s	6	118.6 s	120.0 s	120.9 s
7	162.3 s	161.7 s	161.6 s	7	161.5 s	162.4 s	161.8 s
R_3	56.5 q	56.4 q	56.4 q	R_3	—	56.5 q	56.5 q
8	101.7 d	102.1 d	102.1 d	8	107.4 d	102.0 d	102.2 d
8a	131.7 s	130.9 s	130.5 s	8a	130.7 s	131.5 s	131.1 s
1′	22.8 t	22.8 t	22.9 t	1′	23.3 t	22.8 t	23.0 t
2′	33.5 t	33.5 t	33.6 t	2′	33.7 t	33.6 t	33.5 t
3′	51.9 d	51.9 d	51.9 d	3′	52.2 d	52.0 d	52.1 d
4′	172.0 s	172.2 s	172.1 s	4′	172.3 s	172.1 s	172.2 s
5′	169.1 s	169.2 s	169.2 s	5′	169.5 s	169.2 s	169.4 s
6′	22.4 q	22.5 q	22.4 q	6′	22.6 q	22.5 q	22.5 q

[a] These δ values were assigned by off-resonance decouplings.

^{13}C NMR. The color reactions and IR characteristics were similar to those of **2**. The UV spectrum of **1** in methanol exhibited maxima at 220, 263 (sh), 270 and 420 nm, and showed a bathochromic shift in the presence of base. These data indicated close similarity between structures **1** and **2**. Comparison of the ^1H and ^{13}C NMR spectra of **1** with those of **2** suggested that an aromatic signal in **1** should be located at the 2-position of 5-hydroxy-1,4-naphthoquinone nucleus and the presence of a *N*-acetyl-L-cystein-*S*-yl moiety was also obvious (Tables 2 and 3).

Catalytic hydrogenation of **1** with Raney nickel yielded **2a** and a chromophoric substance **1b**. The molecular formula of **1b** was determined to be $C_{13}H_{12}O_4$ (*m/z* 232 (M^+)) by elemental analysis and EI-MS. The location of the substituents of **1b** and the structure of **1** were determined by the extensive ^{13}C NMR studies including selective decoupling experiments and LPSD methods monitored by ^{13}C-^1H long-range couplings (Table 4). The ^{13}C NMR signals of **1** appeared at almost the same chemical shifts and coupling constants as those of **2**, except for the chemical shifts of signals of C-1, C-2, C-3, C-4 and C-4a, and the multiplicity of the signal of C-8a, changed from doublet in the spectrum of **2** to double doublet in that of **1**. These findings, together with an examination of the diagnostic mass fragmentation (significant peaks at *m/z* 264 and 231 due to the fragments I and II), led to elucidation of the structure **1** for fibrostatin A.

Fibrostatin C (**3**) was obtained as yellowish orange crystals, mp 187~190°C, $[\alpha]_D^{23}$ −93° (*c* 0.51, MeOH). The EI-MS showed the molecular ion peak at *m/z* 409 (M^+). The molecular formula of **3** was determined to be $C_{18}H_{19}NO_6S$ by elemental analysis, high-resolution MS and carbon number in the ^{13}C NMR. The ^{13}C NMR spectrum of **3** indicated the presence of eighteen carbons; six aliphatic carbons, eight aromatic carbons and four carbonyl carbons. The color reactions, IR and UV characteristics were similar to those of **2**. The ^1H and ^{13}C NMR spectra of **3** closely resembled those of **2**, except for the lack of signals attributable to a methyl group at the 3-position (Tables 2 and 3).

Catalytic hydrogenation of **3** gave **2a** and a chromophoric substance **3b**. The molecular formula

Table 4. ^{13}C NMR and LPSD experiments: Assignments and coupling constants for fibrostatins in DMSO-d_6 (100 MHz).

Compound	Carbon No.	δ	Multiplicity with long-range coupling and coupling constant (Hz)						
				(a)	(b)	(c)	(d)	(e)	(f)	
2	1	179.2	d	d	s					^3J=4.0 (8-H)
	2	157.5	m	m						^3J=3.4 (2-OCH_3), 4.7 (3-CH_3)
	R_1 (OCH_3)	60.8	q	q						^1J=147
	3	129.6	q	q						^2J=6.7 (3-CH_3)
	R_2 (CH_3)	8.5	q	q						^1J=130
	4	189.5	q	q						^3J=3.4 (3-CH_3)
	4a	108.4	dd	d	s					^3J=5.4 (5-OH), 6.0 (8-H)
	5	159.1	q	t		s				^2J=4.0 (5-OH), ^3J=4.0 (1′-H)
	6	120.3	quintet	q	t	d				^2J=6.0 (1′-H), ^3J=6.0 (5-OH, 8-H)
	7	161.7	m	m	sextet	br s				^2J=1.3 (8-H), ^3J=3.7 (1′-H, 7-OCH_3)
	R_3 (OCH_3)	56.5	q	q						^1J=146
	8	102.1	d	d						^1J=166
	8a	130.9	d	d	s					^2J=2.7 (8-H)
	1′	22.8	br t	br t						^1J=143
	2′	33.5	tq	tq		br t				^1J=141, ^2J=4.7 (3′-H), ^3J=4.7 (1′-H)
	3′	51.9	br d	br d						^1J=142
	4′	172.2	dq like	dt						^2J=6.0 (3′-H), ^3J=3.0 (2′-H)
	5′	169.2	tq like	dq						^2J=6.0 (6′-H), ^3J=3.0 (3′-H)
	6′	22.5	q	q						^1J=128
1	1	183.2	d	d						^3J=4.0 (8-H)
	2	135.2	dq	dq						^1J=167, ^3J=4.7 (3-CH_3)
	3	147.9	q	q						^2J=6.5 (3-CH_3)
	4	188.8	dq	dq						^3J=4.0 (3-CH_3), 9.4 (2-H)
	4a	109.4	dd	d						^3J=5.4 (5-OH), 6.0 (8-H)
	8a	131.7	dd	dd						^2J=2.0 (8-H), ^3J=4.7 (2-H)
3	1	178.2	dd	dd			d			^3J=4.4 (8-H), 7.7 (3-H)
	2	160.5	quintet	quintet			q			^2J=3.7 (3-H), ^3J=3.7 (2-OCH_3)
	3	109.0	d	d			s			^1J=167
	4	189.7	s	s			s			
	4a	108.3	q like	dd			d			^3J=4.7 (3-H), 6.0 (5-OH, 8-H)
	8a	130.5	d	d						^2J=2.0 (8-H)
4	7	161.5	m	dt	t	d				^2J=1.3 (7-OH, 8-H), ^3J=4.0 (1′-H)
5	1	183.2	d	d	s					^3J=4.7 (8-H)
	2	131.9	dt	dt				t like	d	^1J=168, ^3J=4.4 (3-CH_2OH)
	3	151.5	dt	dt				t	d	^2J=2.0 (2-H), 6.1 (3-CH_2OH)
	4	188.4	d	d				s		^3J=10.1 (2-H)
	4a	109.6	dd	d	s					^3J=5.4 (5-OH), 6.0 (8-H)
	8a	131.5	dd	dd	d			d		^2J=2.0 (8-H), ^3J=4.7 (2-H)
6	1	180.3	d							^3J=4.3 (8-H)
	2	158.5	sextet							^3J=3.7 (2-OCH_3, 3-CH_2OH)
	3	130.5	t							^2J=3.1 (3-CH_2OH)
	4	189.3	t							^3J=4.3 (3-CH_2OH)

(a) Measured after addition of D_2O; (b) measured after addition of D_2O and 8-H was irradiated by LPSD method; (c) measured after addition of D_2O and 1′-H was irradiated by LPSD method; (d) measured after addition of D_2O and 3-H was irradiated by LPSD method; (e) measured after addition of D_2O and 2-H was irradiated by LPSD method; (f) measured after addition of D_2O and 3-CH_2 was irradiated by LPSD method.

of **3b** was determined to be $C_{13}H_{12}O_5$ (*m/z* 248 (M^+)) by elemental analysis and EI-MS. Examination of the ^1H NMR spectrum of **3b** compared with that of **3** suggested that it was a 5-hydroxy-1,4-naphthoquinone derivative having a methyl and two methoxyl groups. Moreover, the UV spectrum of **3b** was similar to that of 6-ethyl-2,7-dimethoxyjuglone[7], whose structure has already been established. The two methoxyl groups in **3** were located at the 2- and 7-positions in the naphthoquinone nucleus, since in the ^{13}C NMR spectrum, determined by LPSD method, all the signals except for the signals at C-1, C-2, C-3, C-4 and C-4a were in good agreement with those observed in the ^{13}C NMR spectrum of **2**. These data were also supported by the ^1H and ^{13}C NMR data (Tables 2 and 3), and an examination of the diagnostic mass fragmentation, which included significant peaks at *m/z* 280 and 247 due to the fragment ions I and II, respectively (Table 1). Consequently, the structure of fibrostatin C was formulated as **3**.

Fibrostatin D (**4**) was obtained as yellowish orange crystals, mp 205~207°C, $[\alpha]_D^{23}$ −62° (*c* 0.51, MeOH). The molecular formula of **4** was determined to be $C_{18}H_{19}NO_8S$ from the elemental analysis, EI-MS and carbon number in ^{13}C NMR. The ^{13}C NMR spectrum of **4** indicated the presence of eighteen carbons; six aliphatic carbons, eight aromatic carbons and four carbonyl carbons. The color reactions, IR and UV spectra were similar to those of the above compounds. The ^1H and ^{13}C NMR spectra of **4** showed almost the same chemical shifts and coupling constants as those of **2**, except for the lack of a signal due to the methoxyl group at the 7-position and the chemical shift of C-8. Therefore, **4** was presumed to be an analog of **2** demethylated at the 7-position.

Catalytic hydrogenation of **4** afforded **2a** and a chromophoric substance **4b**. The molecular formula of **4b** was determined to be $C_{13}H_{12}O_5$ (*m/z* 248 (M^+)) by elemental analysis and EI-MS. Comparison of the ^1H NMR spectrum of **4b** with that of **4** suggested that it was a 5-hydroxy-1,4-naphthoquinone derivative having a phenolic hydroxyl, a methoxyl and two methyl groups. The ^{13}C NMR spectrum of **4** determined by the LPSD method indicated almost the same splitting patterns and coupling constants as those of **2**, except for the signal at δ 161.5 (m, ^2J=1.3 and ^3J=4.0 Hz, C-7), suggesting the presence of a phenolic hydroxyl group at the 7-position. These data were also supported by the ^1H and ^{13}C NMR data (Tables 2 and 3), and examination of the diagnostic mass fragmentation which gave intense peaks at *m/z* 280 and 247 due to the fragment ions I and II, respectively (Table 1). Based on these findings, the structure of fibrostatin D was assigned as formula **4**.

Fibrostatin E (**5**) was obtained as orange-yellow crystals, mp 174~176°C, $[\alpha]_D^{23}$ −86° (*c* 0.5, MeOH). The molecular formula of **5** was determined to be $C_{18}H_{19}NO_6S$ by elemental analysis, fast atom bombardment mass spectrum (FAB-MS) and carbon number in the ^{13}C NMR. The ^{13}C NMR spectrum of **5** indicated the presence of eighteen carbons; six aliphatic carbons, eight aromatic carbons and four carbonyl carbons (Table 3). The color reactions, IR and UV characteristics resembled those of **1**. The ^1H and ^{13}C NMR spectra also closely resembled those of **1**, except for the presence of a signal ascribable to an hydroxymethyl group instead of the methyl group at the 3-position in **1** (Tables 2 and 3). Therefore, **5** was considered to be an hydroxymethylated derivative of **1** at the 3-position.

On catalytic hydrogenation, **5** furnished **2a** and a chromophoric substance **5b**, $C_{13}H_{12}O_4$ (*m/z* 232 (M^+)). The compound **5b** thus obtained was identical with **1b** in mp, elemental analysis and spectral (UV, IR and ^1H NMR) properties. The ^{13}C NMR spectrum of **5** determined by the LPSD method had signals at almost the same chemical shifts and coupling constants as those in the spectrum of **1**, except for the signal at δ 131.9 (dt, ^1J=168 and ^3J=4.4 Hz, C-2), 151.5 (dt, ^2J=2.0 and ^2J=6.1 Hz, C-3) and 188.4 (d, ^3J=10.1 Hz, C-4) (Table 4). These results were also supported by ^1H NMR data

(Table 2) and the examination of the diagnostic mass fragmentation pattern (Table 1). In view of these data, the structure of fibrostatin E was formulated as **5**.

Fibrostatin F (**6**) was obtained as yellowish orange crystals, mp 191~195°C, $[\alpha]_D^{23}$ −91° (*c* 0.51, MeOH). The molecular formula of **6** was determined to be $C_{19}H_{21}NO_9S$ by elemental analysis, EI-MS and carbon number in the ^{13}C NMR. The ^{13}C NMR spectrum of **6** showed the presence of nineteen carbons; seven aliphatic carbons, eight aromatic carbons and four carbonyl carbons. The color reactions, IR and UV characteristics were similar to those of **2**. The ^1H and ^{13}C NMR spectra of **6** also closely resembled those of **2**, except for the signal due to the hydroxymethyl group at the 3-position (Tables 2 and 3).

Catalytic hydrogenation of **6** yielded **2a** and a chromophoric substance **6b**, $C_{14}H_{14}O_5$ (*m/z* 262 (M^+)). The compound **6b** thus obtained was identical with **2b** in mp, elemental analysis and spectral (UV, IR and ^1H NMR) properties. The ^{13}C NMR data of **6** determined by the LPSD method had almost the same chemical shifts and coupling constants as those of **2**, except for the signals at δ 158.5 (sextet, ^3J=3.7 Hz, C-2), 130.5 (t, ^2J=3.1 Hz, C-3) and 189.3 (t, ^3J=4.3 Hz, C-4) (Table 4). These results were further confirmed by ^1H NMR data (Table 2) together with the examination of the diagnostic mass fragmentation pattern (Table 1). Accordingly, the structure of fibrostatin F was elucidated as **6**.

We have applied the LPSD technique to the structural determination of fibrostatins and established the usefulness of this technique. These compounds are the first example of potent prolyl hydroxylase inhibitors containing naphthoquinone chromophores and *N*-acetyl-L-cystein-*S*-yl moieties in the molecule.

Experimental

General

Melting points were determined on a Yanagimoto micro-melting point apparatus and are uncorrected. UV spectra were recorded on a Hitachi 124 or Perkin-Elmer 554 spectrometer, IR spectra on a Hitachi 260-30 spectrometer, mass spectra on a Jeol JMS-O1SG apparatus (EI-MS) or Jeol JMS-DX 300 (FAB-MS). ^1H and ^{13}C NMR spectra were determined on Varian EM-390, XL-100-12 and Jeol JNM GX-400 spectrometers at 90, 100 or 400 MHz (^1H) and 25.2 or 100.40 MHz (^{13}C) using TMS as an internal standard. TLC was carried out on Merck DC-Fertigplatten (Kieselgel 60 F_{254}) and column chromatography was performed on Kieselgel 60 (70~230 mesh ASTM; Merck).

Fibrostatins A (1), B (2), C (3), D (4), E (5) and F (6)

These compounds were isolated from the culture filtrate of *S. catenulae* subsp. *griseospora* No. 23924 as reported previously[1].

Catalytic Hydrogenation of 1, 2, 3, 4, 5 and 6

A solution of each of **1**, **2**, **3**, **4**, **5** and **6** (3.0 g) in MeOH (700 ml) was hydrogenated over Raney nickel (15 g) at 70°C under an initial pressure of hydrogen (100 kg/cm^2) for 5 hours. After filtration, each filtrate was evaporated to dryness. Each residue was dissolved in EtOAc (800 ml) and the resultant solution was washed with 300 ml each portion of H_2O. Each organic layer was separated, dried and concentrated *in vacuo* to give each chromophore of **1b**, **2b**, **3b**, **4b**, **5b** and **6b** as orange red crystals (0.4~1.2 g). Each aqueous layer was concentrated *in vacuo* to a small volume, adjusted to pH 6.0 with 1 N NaOH and applied on a column of Dowex 1-X2 (AcO^- form, 2.4×22 cm) developing with 0.3 N acetic acid. Fractions were monitored with the Rydon-Smith test and TLC using a solvent system of $CHCl_3$ - MeOH (7 : 3). Appropriate fractions were pooled and concentrated under reduced pressure. Each concentrate was applied on a column of the activated carbon (2.4×15 cm)

and the decolorized solution was concentrated to dryness. Crystallization of each residue from a mixture of EtOAc - benzene (1 : 1) gave 200~220 mg each of a colorless solid. Each solid residue was chromatographed on a column of silica gel (1.5×8 cm) with $CHCl_3$ - MeOH (9 : 1→1 : 1), and repeatedly crystallized from EtOAc - benzene, to afford **2a** as colorless needles (each 150~170 mg): MP 122°C; $[\alpha]_D^{23}$ −54.5° (*c* 2.0, H_2O); TLC Rf 0.16 ($CHCl_3$ - MeOH, 7 : 3) and 0.63 ($CHCl_3$ - AcOH, 4 : 1); IR (KBr) cm^{-1} 3340, 1710, 1620, 1560, 1455, 1420, 1380, 1350, 1320, 1260, 1165, 1110, 1050, 1040, 1010, 980, 920, 840, 675; ^1H NMR (100 MHz, DMSO-d_6) δ 1.24 (3H, d, J=7 Hz, CH_3), 1.84 (3H, s, $COCH_3$), 4.19 (1H, dq, J=7 and 7 Hz, CH), 7.66 (1H, br s, COOH), 8.01 (1H, d, J=7 Hz, NH).

Anal Calcd for $C_5H_9NO_3$: C 45.80, H 6.92, N 10.68.
Found: C 44.99, H 6.97, N 10.31.

EI-MS *m/z* 131 (M^+); this product was identified as *N*-acetyl-L-alanine by direct comparison with an authentic sample[4~6].

1b and **5b**: MP 145~149°C; UV λ_{max}^{MeOH} nm ($E_{1cm}^{1\%}$) 219 (1,374), 260 (sh, 688), 265 (705), 283 (sh, 314), 420 (183); $\lambda_{max}^{0.1N\ HCl-90\%\ MeOH}$ nm ($E_{1cm}^{1\%}$) 219 (1,396), 260 (sh, 709), 266 (728), 283 (sh, 333), 420 (188); $\lambda_{max}^{0.1N\ NaOH-90\%\ MeOH}$ nm ($E_{1cm}^{1\%}$) 234 (1,036), 259 (447), 283 (sh, 391), 425 (99), 535 (181); IR (KBr) cm^{-1} 3450~3400, 3000~2900, 1660, 1630, 1605, 1570, 1490, 1420, 1370, 1330, 1310, 1275, 1240, 1200, 1165, 1140, 1060, 1000, 910, 870, 810, 800, 700, 620; ^1H NMR (400 MHz, DMSO-d_6) δ 2.05 (3H, s, 6-CH_3), 2.09 (3H, d, J=1.6 Hz, 3-CH_3), 3.95 (3H, s, 7-OCH_3), 6.87 (1H, q, J=1.6 Hz, 2-H), 7.06 (1H, s, 8-H), 12.31 (1H, s, disappeared on D_2O, 5-OH).

Anal Calcd for $C_{13}H_{12}O_4$: C 67.23, H 5.21.
Found: C 66.99, H 5.27.

EI-MS *m/z* 232 (M^+); **1b** was shown to be identical with **5b** by direct comparison of their physical and spectral data.

2b and **6b**: MP 156~159°C; UV λ_{max}^{MeOH} nm ($E_{1cm}^{1\%}$) 218 (1,310), 260 (sh, 677), 264 (713), 308 (371), 420 (162); $\lambda_{max}^{0.1N\ HCl-90\%\ MeOH}$ nm ($E_{1cm}^{1\%}$) 218 (1,294), 259 (sh, 674), 264 (722), 308 (365), 425 (165); $\lambda_{max}^{0.1N\ NaOH-90\%\ MeOH}$ nm ($E_{1cm}^{1\%}$) 234 (1,111), 266 (416), 295 (sh, 294), 420 (79), 530 (166); IR (KBr) cm^{-1} 3500~3400, 2960, 1670, 1630, 1600, 1490, 1455, 1420, 1380, 1330, 1290, 1230, 1220, 1150, 1110, 1080, 1020, 980, 920, 870, 820, 790, 705; ^1H NMR (400 MHz, DMSO-d_6) δ 1.95 (3H, s, 3-CH_3), 2.05 (3H, s, 6-CH_3), 3.95 (3H, s, 7-OCH_3), 4.03 (3H, s, 2-OCH_3), 7.10 (1H, s, 8-H), 12.51 (1H, s, disappeared on D_2O, 5-OH).

Anal Calcd for $C_{14}H_{14}O_5$: C 64.12, H 5.38.
Found: C 63.93, H 5.52.

EI-MS *m/z* 262 (M^+); **2b** was shown to be identical with **6b** by direct comparison of their physical and spectral data.

3b: MP 210~212°C; UV λ_{max}^{MeOH} nm ($E_{1cm}^{1\%}$) 218 (1,417), 256 (sh, 708), 262 (739), 305 (441), 425 (188); $\lambda_{max}^{0.1N\ HCl-90\%\ MeOH}$ nm ($E_{1cm}^{1\%}$) 218 (1,417), 256 (sh, 736), 263 (771), 307 (448), 425 (195); $\lambda_{max}^{0.1N\ NaOH-90\%\ MeOH}$ nm ($E_{1cm}^{1\%}$) 233 (1,224), 260 (409), 285 (343), 305 (sh, 329), 535 (203); IR (KBr) cm^{-1} 3450~3400, 2930, 1680, 1640, 1600, 1485, 1420, 1380, 1350, 1310, 1260, 1250, 1220, 1210, 1140, 1120, 1015, 980, 960, 920, 870, 850, 790, 710; ^1H NMR (400 MHz, DMSO-d_6) δ 2.06 (3H, s, 6-CH_3), 3.87 (3H, s, 2-OCH_3), 3.95 (3H, s, 7-OCH_3), 6.24 (1H, s, 3-H), 7.13 (1H, s, 8-H), 12.63 (1H, s, disappeared on D_2O, 5-OH).

Anal Calcd for $C_{13}H_{12}O_5$: C 62.90, H 4.87.
Found: C 62.96, H 5.10.

EI-MS *m/z* 248 (M^+).

4b: MP 263~266°C; UV λ_{max}^{MeOH} nm ($E_{1cm}^{1\%}$) 218 (1,288), 267 (761), 303 (407), 425 (170); $\lambda_{max}^{0.1N\ HCl-90\%\ MeOH}$ nm ($E_{1cm}^{1\%}$) 218 (1,314), 267 (791), 306 (391), 425 (174); $\lambda_{max}^{0.1N\ NaOH-90\%\ MeOH}$ nm ($E_{1cm}^{1\%}$) 230 (1,038), 292 (970), 440 (149), 535 (143); IR (KBr) cm^{-1} 3430, 2950, 1655, 1620, 1595, 1490, 1450, 1430, 1360, 1310, 1290, 1220, 1130, 1100, 1080, 1020, 940, 890, 865, 830, 785, 760, 710, 685; ^1H NMR (400 MHz, DMSO-d_6) δ 1.92 (3H, s, 3-or 6-CH_3), 2.01 (3H, s, 3- or 6-CH_3), 3.99 (3H, s, 2-OCH_3), 6.99 (1H, s, 8-H), 10.94 (1H, s, disappeared on D_2O, 7-OH), 12.64 (1H, s, disappeared on D_2O, 5-OH).

Anal Calcd for $C_{13}H_{12}O_5$: C 62.90, H 4.87.
Found: C 63.03, H 4.99.

EI-MS *m/z* 248 (M^+).

X-Ray Crystallographic Analysis of **2b**

The crystal belongs to the triclinic $P\bar{1}$ with cell dimensions of $a=10.180(3)$, $b=8.037(3)$, $c=7.887(3)$ Å, $\alpha=94.65(3)$, $\beta=105.45(2)$, $\gamma=93.34(3)^\circ$ and $V=617.8(3)$ Å^3. The calculated density is 1.41 g·cm^{-3}. Out of 1630 independent reflections measured on a Rigaku AFC-5 diffractometer using MoKα radiation ($\lambda=0.7107$ Å), 1263 had $F_o \geqq 2\sigma$ (F_o) and therefore were used in the calculations for structural determination. The structure was solved by direct methods using the program MULTAN[8], and refined to an R of 0.086 by the program X-ray 76[9].

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