DEPHOSTATIN, A NOVEL PROTEIN TYROSINE PHOSPHATASE INHIBITOR PRODUCED BY *Streptomyces*

II. STRUCTURE DETERMINATION

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Dephostatin, a novel tyrosine phosphatase inhibitor, was isolated from the culture broth of *Streptomyces* sp. MJ742-NF5. The structure was elucidated to be 2-(*N*-methyl-*N*-nitroso)hydroquinone by spectral and chemical analyses of dephostatin and its derivatives.

Dephostatin (**1**), a novel tyrosine phosphatase inhibitor, was isolated from the culture broth of *Streptomyces* sp. MJ742-NF5. The taxonomy, fermentation, isolation, and biological activity of **1** was reported previously[1]. In this paper, we describe the physico-chemical properties and the structure of dephostatin.

1 was obtained as a colorless powder. The physico-chemical properties of **1** are summarized in Table 1. The FAB-MS spectrum of **1** afforded *m/z* 169 $(M+H)^+$ and 167 $(M-H)^-$. The UV spectra of **1** showed absorption maxima at 305 nm (ε 3,280) in MeOH, 305 nm (ε 3,450) in 0.1 N HCl-MeOH,

Fig. 1. Structure of dephostatin (**1**).

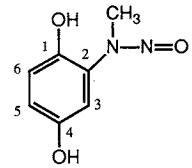


Table 1. Physico-chemical properties of dephostatin (**1**) and **2**.

	Dephostatin (**1**)	**2**
Appearance	Colorless powder	Colorless needles
MP	104~107°C	131°C
Molecular formula	$C_7H_8N_2O_3$	$C_7H_{11}NO_2$
FAB-MS (Positive) *m/z*	169 $(M+H)^+$	
(Negative) *m/z*	167 $(M-H)^-$	
HREI-MS Found:		141.0794
Calcd:		141.0789
Elemental analysis Found:	C 49.13, H 4.64, N 13.80%	C 59.41, H 7.89, N 9.64, O 22.63%
Calcd:	C 50.00, H 4.80, N 16.66%	C 59.56, H 7.85, N 9.92, O 22.67%
UV λ_{max}^{MeOH} nm (ε)	305 (3,280)	290 (16,215)
$\lambda_{max}^{MeOH-HCl}$ nm (ε)	305 (3,450)	280 (14,100)
$\lambda_{max}^{MeOH-NaOH}$ nm (ε)	314 (3,175)	290 (16,215)
IR ν_{max} (KBr) cm^{-1}	3235, 1540, 1475, 1420, 1355, 1275, 1235, 1080, 850, 810, 790, 625	3345, 1580, 1540, 1245, 1210, 1100, 1080, 852, 800
Rf value[a] $CHCl_3$-MeOH (5:1)	0.56	0.21
Solubility Soluble:	MeOH, acetone	MeOH, acetone
Insoluble:	H_2O, *n*-hexane	H_2O, *n*-hexane

[a] Silica gel TLC (Merck $60F_{254}$).

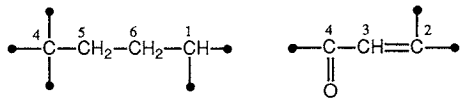
Table 2. ^{13}C and ^{1}H NMR assignments for dephostatin (**1**) and **2**.

Dephostatin (**1**)			**2**		
No.	^{13}C[a]	^{1}H[b]	No.	^{13}C[a]	^{1}H[b]
1, 4	144.5, 151.5		1	67.8	4.41 (1H, dd, J=4.0, 11.2 Hz)
2	131.2		2	167.8	
3, 5, 6	113.8, 117.3, 118.4	6.80~6.98 (3H, m)	3	94.2	4.76 (1H, s)
N-CH_3	34.5	3.35 (3H, s)	4	194.2	
1,4-OH		8.18 (1H, br s),	5	35.9	2.20~2.23 (2H, m)
		8.41 (1H, br s)	6	33.0	1.85 (1H, m),
					2.16 (1H, m)
			N-CH_3	29.2	2.76 (3H, d)
			OH, NH		4.97 (1H, br s),
					6.36 (1H, br s)

[a] Measured in acetone-d_6 at 67.5 MHz; chemical shifts in ppm from TMS.
[b] Measured in acetone-d_6 at 270 MHz; chemical shifts in ppm from TMS.

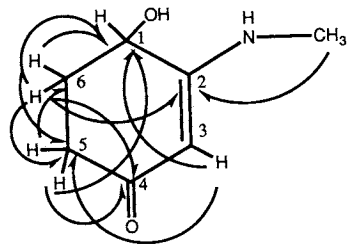
Fig. 2. Partial structures of **2** from spin decoupling (a) and ^{1}H NMR, ^{13}C NMR, and UV spectra (b).

●: Nonproton atom.



(a) (b)

Fig. 3. ^{1}H-^{13}C correlation by HMBC experiment of **2**.



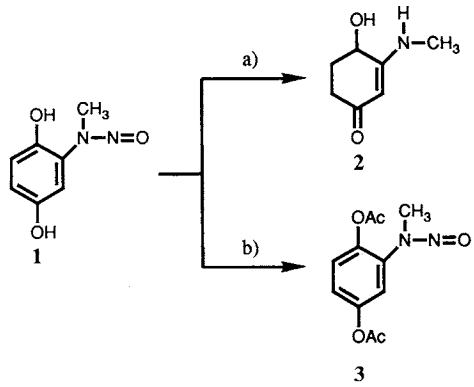
and 314 nm (ε 3,175) in 0.1 N NaOH-MeOH. Its IR spectrum (KBr) revealed absorptions at 3235, 1540, 1475, 1420, 1355, 1275, 1235, 1080, 850, 810, 790, and 625 cm^{-1}. The ^{1}H NMR spectrum of **1** in acetone-d_6 at 270 MHz showed eight protons; a signal at δ 3.35 (3H, s), aromatic protons at δ 6.80~6.98 (3H, m), and the presence of two broad signals at δ 8.18 (1H, br s) and 8.41 (1H, br s), which disappeared by the addition of deuterium oxide. The ^{13}C NMR spectrum of **1** revealed the presence of 7 carbon atoms (δ 34.5, 113.8, 117.3, 118.4, 131.2, 144.5, and 151.5).

1 was unstable in solvents such as MeOH and CH_3CN; especially, it easily decomposed in acidic media. Then, **1** was converted to **2** by catalytic hydrogenation with palladium on carbon in EtOH.

2 was obtained as colorless needles, and shown to be more stable than **1**. The molecular formula of **2** was determined to be $C_7H_{11}NO_2$ from the result of HREI-MS (Found: m/z 141.0794, Calcd: m/z 141.0789) and elemental analysis (Found: C 59.41, H 7.89, N 9.64, O 22.63, Calcd: C 59.56, H 7.85, N 9.92, O 22.67). The absorption band at 3345 cm^{-1} in the IR spectrum of **2** suggested the presence of hydroxyl and amino groups. UV absorption of **2** at 290 nm (ε 16,215) suggested the presence of α,β-unsaturated ketone chromophore. The physico-chemical properties and NMR data of **2** are summarized in Tables 1 and 2, respectively. The ^{1}H and ^{13}C NMR spectra of **2** revealed the presence of 11 protons and 7 carbon atoms in the molecule. The partial structure (Fig. 2a) was obtained from a spin-decoupling experiment. The ^{1}H NMR, ^{13}C NMR, and UV spectra afforded another partial structure (Fig. 2b). Moreover, the partial structures and unsaturation number suggested that **2** was a cyclohexenone derivative. The structure of **2** was defined by heteronuclear multiple-bond correlation (HMBC) spectrum (Fig. 3). The

methyl protons (δ_H 2.76) showed their coupling to C-2, suggesting that a methylamino group attaches to C-2. The olefinic proton of 3-CH (δ_H 4.76) showed coupling to C-1 and C-5. The methylene protons of 6-CH_2 (δ_H 1.85, 2.15) coupled to C-1, C-2, C-4, and C-5. The methylene protons of 5-CH_2 (δ_H 2.20~2.23) coupled to C-1, C-4, and C-6. From these data, the structure of **2** was determined to be as shown in Fig. 3.

Fig. 4. Scheme for hydrogenation (a) and acetylation (b) of dephostatin (**1**). Explanations in text.



a) H_2, 10% Pd-C-EtOH; b) acetic anhydride-pyridine.

Based on the structure of **2**, **1** was elucidated to be a 1,2,4-trisubstituted benzene derivative. Acetylation of **1** afforded dephostatin diacetate (**3**) in high yield (Fig. 4). In particular, the ^1H chemical shift of the two acetyl groups (δ_H 2.23, 2.32) and strong IR absorption band at 1765 cm^{-1} are characteristic of phenolic acetate. The structure of **2** and **3** indicated that **1** possessed two phenolic OH groups at the 1,4 positions and *N*-methyl group at the 2 position. Moreover, FAB-MS [*m/z* 169 $(M+H)^+$ and 139 $(M+H-NO)^+$] and IR (1475 cm^{-1}) spectra showed that **1** possessed a nitrosoamine group.[2] The FAB-MS [*m/z* 253 $(M+H)^+$ and 223 $(M+H-NO)^+$] and IR (1460 cm^{-1}) spectra of **3** showed that **3** would also possess a nitrosoamine group. The FAB-MS and IR spectra of **2** did not show the ion peak $(M+H-NO)^+$ and absorption peak arising from a nitrosoamine group. These results suggested that the reductive cleavage of *N*-nitroso bond occured in **2** and that **1** possessed an *N*-methyl-*N*-nitroso group at the 2 position.

Thus, the structure of dephostatin (**1**) was confirmed to be 2-(*N*-methyl-*N*-nitroso)hydroquinone (Fig. 1). The ^1H and ^{13}C NMR data of **1** are shown in Table 2.

Experimental

General

MP's were determined with a micro mp apparatus, MP-3 (Yanagimoto), and were uncorrected. UV spectra were measured on a Hitachi U-3210 spectrophotometer. IR spectra were measured with a Hitachi I-5020 FT-IR spectrometer. FAB-MS and EI-MS were taken by a JEOL JMS-SX 102 and a Hitachi M-80H mass spectrometer, respectively. NMR spectra were recorded with JEOL JNM-EX 270 and JEOL JNM-GX400 NMR spectrometers.

Hydrogenation of 1

A solution of **1** (7.0 mg, 0.042 mmol) in EtOH (3 ml) was hydrogenated over 10% Pd-C (2 mg) under an atmosphere of hydrogen at room temperature for 4 hours. The mixture was filtered, and the filtrate was concentrated *in vacuo*. The oily residue was chromatographed on a silica gel column (Merck silica gel 60), eluting with $CHCl_3$-MeOH (5:1). Fractions containing the major product were concentrated *in vacuo*, and the residue was applied on Sephadex LH-20. After elution with MeOH, the pure fractions were concentrated *in vacuo* to give colorless needles of **2** (6.0 mg, quant.).

Acetylation of 1

A solution of **1** (10 mg, 0.060 mmol) in pyridine (3 ml) and acetic anhydride (1 ml) was stirred at room temperature for 5 hours. The mixture was diluted with $CHCl_3$ and washed successively with 1 N hydrochloric

acid, water, and brine. The organic layer was dried and concentrated *in vacuo* to give an oily residue. The oily residue was chromatographed on a silica gel column, eluting with $CHCl_3$-MeOH (5:1). Fractions containing the major product were concentrated *in vacuo* and the residue was applied on Sephadex LH-20. After elution with MeOH, the pure fraction was concentrated *in vacuo* to give **3** (14.5 mg, 97%) as pale yellow crystals: MP 86°C; FAB-MS (positive) *m/z* 253 $(M+H)^+$, 223 $(M+H-NO)^+$; UV λ_{max}^{MeOH} nm (ε) 255 (39,525); ^1H NMR ($CDCl_3$, 270 MHz) δ 2.23 (3H, s), 2.32 (3H, s), 3.05 (3H, s), 7.18~7.28 (3H, m); IR ν_{max} cm^{-1} 1765, 1505, 1460, 1170.

References

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