Reinvestigation of Structure of Porritoxin, a Phytotoxin of *Alternaria porri*

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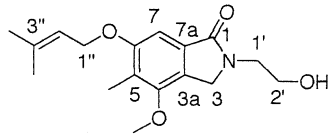
The structure of porritoxin, a phytotoxin of *Alternaria porri*, was reinvestigated by detailed 2D NMR analysis including $^{1}H-^{13}C$ and $^{1}H-^{15}N$ HMBC experiments. The structure of porritoxin was determined to be 2-(2′-hydroxyethyl)-4-methoxy-5-methyl-6-(3″-methyl-2″-butenyloxy)-2,3-dihydro-1*H*-isoindol-1-one (**1**). Thus our previous proposed structure, 8-(3′,3′-dimethylallyloxy)-10-methoxy-9-methyl-1*H*-3,4-dihydro-2,5-benzoxazocin-6(5*H*)-one (**2**), is incorrect.

The fungus *Alternaria porri* (Ellis) Ciferri, the causal fungus of black spot disease in stone-leek and onion, produced tentoxin,[1] silvaticol,[2] and porritoxinol.[3] In 1992, we isolated a new phytotoxin named porritoxin (**2**) from the culture liquid of *Alternaria porri* (Ellis) Ciferri.[4] Comparison of the ^{1}H and ^{13}C NMR data of porritoxin (**2**) with zinniol (**3**)[5,6] led us to suspect that **2** is related to **3**. Porritoxin (**2**) resembled zinniol (**3**), except for the disappearance of one of two CH_2OH groups in **3** (δ 4.61, 4.69), the remarkable lower shift of an aromatic proton (δ 7.07) (δ 6.66 in **3**), the existence of two more methylene groups (δ 3.76 and 3.92), and an amide proton (δ 3.17). The NMR data suggested to us that the two CH_2OH groups of **3** are substituted with CONH and $CH_2OCH_2CH_2$ groups to form a bicyclic structure, porritoxin, defined as **2**. The striking lower shift of an aromatic proton in **2** could be attributed to the anisotropy of the carbonyl group located at a position *peri* to the aromatic hydrogen in **2**. Porritoxin was therefore assigned as 8-(3′,3′-dimethylallyloxy)-10-methoxy-9-methyl-1*H*-3,4-dihydro-2,5-benzoxazocin-6(5*H*)-one (**2**).[4] In 1993, Ayer and Miao reported the secondary metabolite named stachybotramide (**4**), having an isoindole moiety, from the fungus *Stachybotrys cylindrospora*.[7] Comparison of ^{1}H and ^{13}C NMR and IR spectral data of porritoxin with **4** led to reassignment of the methoxy (δ 56.6) and allylic methylene (δ 61.8) signals in **3** and C-1 (δ 50.1) and C-1′ (δ 65.8) in **2**.[8] They pointed out that the structure of porritoxin for **2** may be incorrect and porritoxin may possess an isoindole structure.[8] We have now reinvestigated the structure of porritoxin by detailed 2D NMR analysis including $^{1}H-^{13}C$ and $^{1}H-^{15}N$ HMBC experiments. This paper reports the revised structure of porritoxin (**1**).



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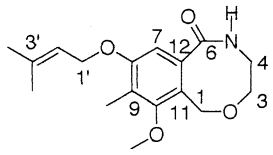

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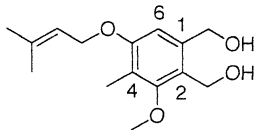

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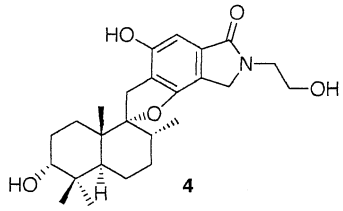

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As previously reported, porritoxin possesses the molecular formula $C_{17}H_{23}O_4N$.[4] The ^{13}C NMR, DEPT, and $^{1}H-^{13}C$ HSQC spectra revealed the presence of four methyls, four methylenes, two methines, seven quaternary carbons, and 22 carbon-bonded protons. Of the four oxygen functions, one was ascribed to a primary hydroxy group (δ_C 60.4, δ_H 3.60, ν_{max} 3340 and 1010 cm^{-1}) and one was ascribed to an amide carbonyl group (δ_C 168.4, ν_{max} 1670 and 1645 cm^{-1}). From consideration of the high-resolution EIMS, ^{13}C NMR (δ_C 60.2, 154.2, 66.3, and 158.6), and IR (ν_{max} 1125, 1100 cm^{-1}) data, the remaining two oxygens were attributed to ether groups. A non-carbon-bonded proton at δ_H 4.82 was assigned to a hydroxy group at C-2′ using double quantum filtered (DQF)-COSY and NOESY data. Furthermore, no nitrogen-bonded proton was observed in the ^{1}H NMR (DMSO-d_6 solution) spectrum or by $^{1}H-^{15}N$ HSQC experiment.[9,10] This evidence was in direct agreement with the designation of structure **1** for porritoxin rather than **2**.

The $^{1}H-^{13}C$ HMBC correlations of H-7 (δ_H 6.97) to C-1 (δ_C 168.4), H-3 (δ_H 4.63) to C-1, H-3 to C-3a (δ_C 133.2), and H-3 to C-7a (δ_C 124.2) and $^{1}H-^{15}N$ HMBC[9] correlation of H-3 to N-2 (δ_N 120.0) revealed the 2,3-dihydro-1*H*-isoindol-1-one ring. The hydroxyethyl partial structure was characterized by DQF-COSY and NOESY data. The HMBC correlations H-1′ (δ_H 3.54) to C-1, H-1′ to C-3 (δ_C 50.0), H-1′ to N-2, and H-2′ (δ_H 3.60) to N-2 clearly indicated that the hydroxyethyl group was attached to nitrogen at position 2 in the isoindole ring. The other structural parts were also

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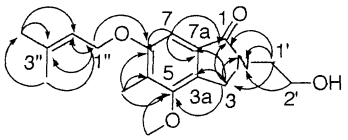

1

Figure 1. Key $^1H-^{13}C$ HMBC and $^1H-^{15}N$ HMBC correlations of porritoxin (**1**).

confirmed by DQF-COSY, NOESY, and $^1H-^{13}C$ HMBC (Figure 1) data. Therefore, porritoxin was unambiguously determined to be 2-(2′-hydroxyethyl)-4-methoxy-5-methyl-6-(3″-methyl-2″-butenyloxy)-2,3,-dihydro-1*H*-isoindol-1-one (**1**).

Experimental Section

General Experimental Procedures. The UV−vis spectra were recorded with a Shimadzu UV-2100 spectrophotometer. EIMS spectra were recorded using a Hitachi M-80B mass spectrometer. The ^1H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectra were measured with a Varian UNITY INOVA 500 spectrometer in DMSO-d_6 with TMS as an internal standard. The ^{15}N (50 MHz) chemical shift was recorded by $^1H-^{15}N$ gradient (g) HMBC experiment.[9] DQF-COSY, NOESY (mixing time 1.3 s), $^1H-^{13}C$ gHSQC ($^1J_{CH}$ = 142 Hz) and $^1H-^{13}C$ gHMBC ($^nJ_{CH}$ optimized for 8 Hz), and $^1H-^{15}N$ gHSQC ($^1J_{CH}$ = 90 Hz) and $^1H-^{15}N$ gHMBC ($^nJ_{NH}$ optimized for 5 Hz) spectra were acquired using the standard Varian pulse programs, and the software used to obtain 2D spectra was from Varian, version 6.1A.

Fungus. The strain of *Alternaria porri* used in this experiment was purchased from IFO (Institute for Fermentation, Osaka), strain number 9762.

Extraction and Isolation of Porritoxin. *A. porri* was cultured in Richards medium. The composition of the medium was as follows (g/L): sucrose (50.0), KNO_3 (10.0), KH_2PO_4 (5.0), $MgSO_4$ (2.5), $FeCl_3$ (0.01). The medium was sterilized in an autoclave at 121 for 70 min. After culturing for 35 days, 10 g of Amberlite XAD-7 was added to 1 L of culture liquid, which was stirred overnight. The adsorbates were eluted with Me_2CO. The Me_2CO extract was dissolved in C_6H_6 and washed with 0.1 M $NaHCO_3$, and the solvent was evaporated to dryness. The extract was subjected to preparative TLC (Merck silica gel 60 PF_{254}) in $C_6H_6-Me_2CO-HOAc$ (60:40:1). The fraction at R_f 0.59 was further purified by HPLC using YMC S-343 (Yamamura Chemical Labs) with a solvent of H_2O-CH_3CN (3:2) and gave needles of mp 115−116 °C in a yield of 4.2 mg from 8 L of culture medium.

Table 1. NMR Assignments for Porritoxin (**1**) in DMSO-d_6

position	δ_H	δ_C	δ_N	COSY
1		168.4		
2			120.0	
3	4.63	50.0		
3a		133.2		
4		154.2		
4-OMe	3.87	60.2		
5		121.8		
5-Me	2.09	10.6		
6		158.6		
7	6.97	101.5		
7a		124.2		
1′	3.54 (t 5.5 Hz)	45.8		
2′	3.60 (m 5.0, 5.5 Hz)	60.4		H-2′-OH
2′-OH	4.82 (t 5.0 Hz)			H-2′
1″	4.58 (d 6.5 Hz)	66.3		H-2″, H-3″-Me, H-4″
2″	5.42 (t 6.5 Hz)	121.1		H-1″, H-3″-Me, H-4″
3″		138.2		
3″-Me	1.72	19.3		H-1″, H-2″
4″	1.75	26.6		H-1″, H-2″

Porritoxin (1): needles, 115−116 °C, IR (KBr) ν_{max}, 3340, 1010 (OH), 2959, 1450 (Me, CH_2), 1670, 1645 (amide), 1600, 765, 700 (phenyl), 1125, 1100 (ether oxygen) cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 215 (4.73), 255 (4.07), 292 (3.60), nm; ^1H (500 MHz), ^{13}C (125 MHz), and ^{15}N (50 MHz) NMR data in Table 1; EIMS *m/z* 305 $[M]^+$, 237 $[M - C_5H_9 + H]^+$, 222 $[237 - Me]^+$, 206 $[237 - OMe]^+$, 162 $[206 - C_2H_4OH + H]^+$, 91 $[C_7H_7]^+$, and 69 $[(Me)_2{=}CHCH_2]^+$; HREIMS *m/z* 305.1652 (calcd for $C_{17}H_{23}O_4N$ 305.1626).

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(10) No $^1H-^{15}N$ correlation signal was observed in the $^1H-^{15}N$ HSQC spectrum.

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