PHTHOXAZOLIN A, A SPECIFIC INHIBITOR OF CELLULOSE BIOSYNTHESIS FROM MICROBIAL ORIGIN

II. ISOLATION, PHYSICO-CHEMICAL PROPERTIES, AND STRUCTURAL ELUCIDATION

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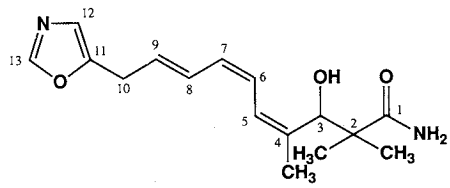
Phthoxazolin A is a new inhibitor of cellulose biosynthesis produces by *Streptomyces* sp. OM-5714. The active compound was isolated, and the structure was elucidated by spectrometric analyses.

Phthoxazolin A (**1**, Fig. 1) is a new metabolite of *Streptomyces* discovered in our screening program for inhibitors of cellulose biosynthesis. The discovery, taxonomy of the producing-microorganism, fermentation, and biological properties of **1** were reported previously.[1,2] This paper describes the isolation, physico-chemical properties, and structure elucidation of **1**.

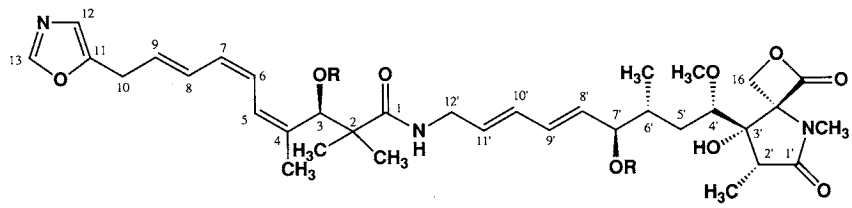
Isolation

Fermentation for production of **1** was carried out in a 30-liter fermentor as described.[2] The isolation procedures for **1** are outlined in Fig. 2. The whole cultured broth (18 liters) was extracted with ethyl acetate. The organic layer was concentrated under reduced pressure. The oily residue (*ca.* 20 g) was subjected to

Fig. 1. Structures of phthoxazolin A (**1**), oxazolomycin (**2**), and diacetyloxazolomycin (**3**).



Phthxazolin A (**1**)



Oxazolomycin (**2**) R=H
Diacetyloxazolomycin (**3**) R=$COCH_3$

two successive column chromatographies on SiO_2, which were eluted first with $CHCl_3$-CH_3OH (100 : 1 ~ 10 : 1), and secondarily with benzene - acetone (100 : 1 ~ 1 : 1). The resulting oily material (157 mg) was further purified by HPLC: column, YMC pack A-303; column size, i.d. 4.65 × 250 mm; solvent, a linear gradient of CH_3CN-H_2O (2 : 8→9 : 1); flow rate, 1.0 ml/minute; detection by UV at 210 nm and 275 nm. The purified **1** was obtained as pale yellow powder (17 mg). It gave a single peak on analytical HPLC as detected both at 210 and 275 nm.

Physico-chemical Properties

The physico-chemical properties of **1** is summarized in Table 1. Compound **1**, mp 58 ~ 62°C, is soluble in methanol, ethanol, and chloroform, but is insoluble in *n*-hexane and water. The UV spectrum shows the absorption maximum at 275 nm (Fig. 3) with two shoulders characteristic of a triene moiety. The molecular formula of **1** was established to be $C_{16}H_{22}N_2O_3$ by HREI-MS, whose molecular ion was shown at *m/z* 290.1630 (M^+, calcd for $C_{16}H_{22}N_2O_3$, 290.1630). A survey based on these data revealed that none of known microbial metabolites shared the physico-chemical properties of **1**. Therefore, it was concluded that **1** was a new compound. The name phthoxazolin was given

Fig. 2. Isolation procedures for **1**.

Whole broth (20 liters)
↓ extracted with EtOAc
EtOAc layer
↓ concentrated *in vacuo*
Oily material (20 g)
↓ silica gel column chromatography ($CHCl_3$ - MeOH = 100 : 1 ~ 10 : 1)
Active fraction
↓ concentrated *in vacuo*
Crude powder (1.3 g)
↓ silica gel column chromatography (benzene - acetone = 100 : 1 ~ 1 : 1)
Oily material (157 mg)
↓ HPLC
column: ODS
solvent: 30% CH_3CN
detection: 275, 210 nm
Pale yellow powder (17 mg)

Fig. 3. UV spectrum of **1** (methanol).

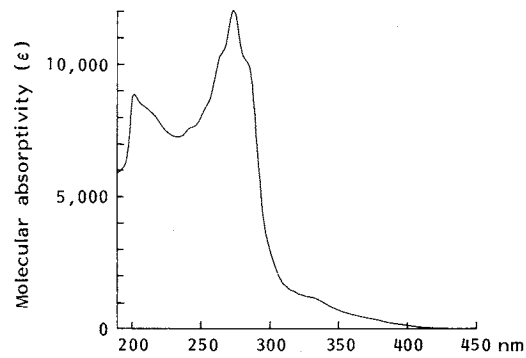


Table 1. Physico-chemical properties of **1**.

Appearance	Pale yellow powder
Nature	Neutral lipophilic compound
MP (°C)	58 ~ 62
$[\alpha]_D^{18}$	+37.4° (*c* 1.0, CH_2Cl_2)
HREI-MS (*m/z*) Found:	290.1630 (M^+)
Calcd:	290.1630
Molecular formula	$C_{16}H_{22}N_2O_3$
UV λ_{max}^{MeOH} nm (ε)	203 (8,900), 215 (sh, 8,200), 243 (sh, 7,600), 253 (sh, 8,200), 265 (sh, 10,300), 275 (12,000), 285 (sh, 10,000), 330 (sh, 1,000)
IR ν_{max} (KBr) cm^{-1}	3360, 2900, 1650, 1580, 1500, 1450, 1370
Color reaction Positive	H_2SO_4, iodine, phosphomolybdate
Negative	Ninhydrin, Molisch, Elson-Morgan

Fig. 4. ^{1}H NMR spectrum of **1** (400 MHz, $CDCl_3$).

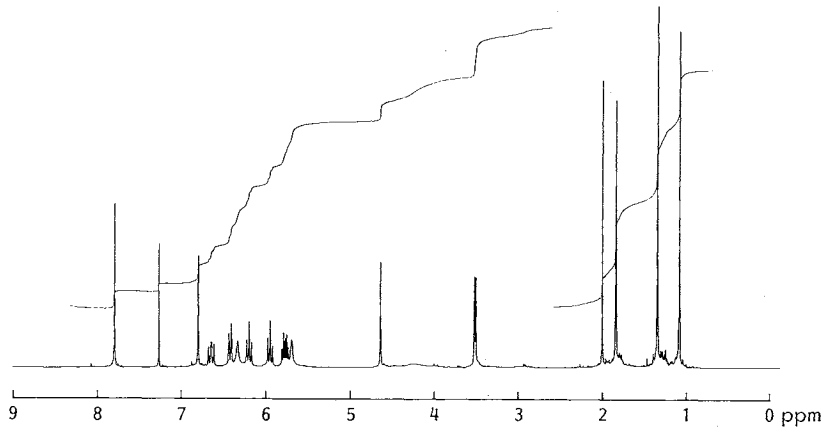
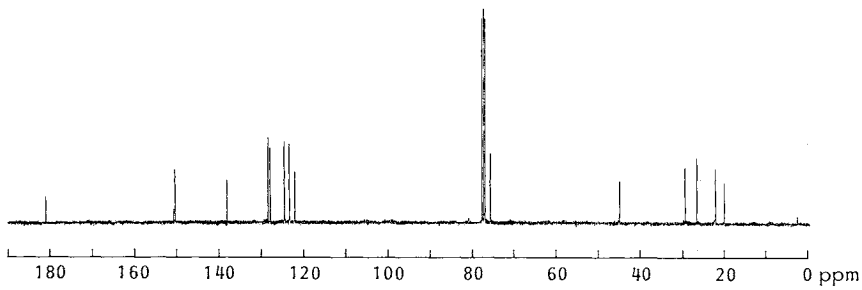


Fig. 5. ^{13}C NMR spectrum of **1** (100 MHz, $CDCl_3$).



because of its selective antimicrobial activity against *Phytophthora* spp.,[2] and an oxazole moiety.

Structural Elucidation

The ^{1}H NMR and ^{13}C NMR spectra of **1** are shown in Figs. 4 and 5, respectively. Chemical shifts in the NMR spectra are shown in Table 2. ^{13}C-^{1}H COSY revealed four quarternary carbons, seven sp^2 methines, one oxy methine, one methylene, three methyls, and three active hydrogens.

Partial structure A was deduced from ^{1}H-^{1}H COSY and long-range ^{13}C-^{1}H COSY (Fig. 6). ^{1}H-^{1}H COSY showed the coupling among neighboring olefinic protons (5-H to 9-H), and 10-H_2. The hydroxy proton (3-OH, δ 4.02) had a coupling with 3-H (δ 4.64). Two methyl protons (2-CH_3, δ 1.08, 1.35) had the ^{13}C-^{1}H long-range couplings with C-1 (δ 181.2), C-2 (δ 44.6), C-3 (δ 75.3), which suggested the alignment of C-1, C-2, C-3, and 2-CH_3. The long-range coupling of C-3/5-H (δ 6.41), C-4 (δ 138.3)/4-CH_3 (δ 1.84), 4-CH_4 (δ 19.4)/3-H (δ 4.64), and 4-CH_3/5-H revealed the arrangement of C-4 and 4-CH_3. IR spectrum of **1** showed amide carbonyl absorption at 1650 cm^{-1} and an amino residue (δ 5.70, 6.33) was suggested to connect C-1.

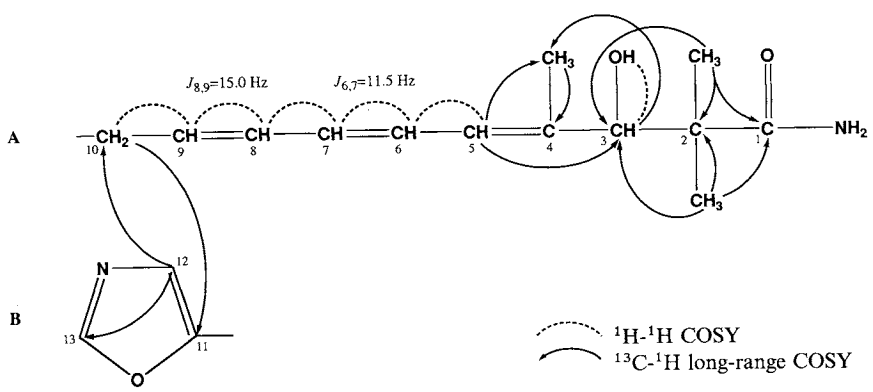
According to the molecular formula, seven atoms remain undefined; three carbons, two hydrogens, one nitrogen, and one oxygen. These atoms were reasoned to construct a monosubstituted oxazole ring (partial structure **B**), based on the chemical shifts of NMR assignable to two aromatic methine and one aromatic quarternary carbon, and because of the unsaturation index of 3 for this moiety. The long-range

Table 2. ^{1}H and ^{13}C NMR spectral data of **1** and **3**.

No.	**1**		**3**[4]
	^{13}C chemical shift	^{1}H chemical shift	^{1}H chemical shift
1	181.2 s		
1-NH_2(NH)		5.70 br s, 6.33, br s	6.06 br t (5.4)
2	44.6 s		
2-Me	21.6 q	1.08 s	1.23 s
2-Me	26.1 q	1.35 s	1.23 s
3	75.3 d	4.64 d (5.8)	5.85 s
3-OH(OAc)		4.02 d (5.8)	2.07 s
4	138.3 s		
4-Me	19.4 q	1.84 s	1.77 br s
5	124.8 d	6.41 d (11.5)	6.48 br d (11.3)
6	123.7 d	6.10 dd (11.5, 11.5)	6.35 dd (11.3, 11.3)
7	128.1 d	5.95 dd (11.5, 11.5)	6.00 dd (11.3, 11.3)
8	128.2 d	6.64 dd (11.5, 15.0)	6.62 br dd (11.3, 15.3)
9	128.6 d	5.77 dt (7.0, 15.0)	5.59 dt (7.2, 15.3)
10	29.0 t	3.51 d (7.0)	3.52 d (7.2)
11	150.7 s		
12	122.4 d	6.80 s	6.80 br s
13	150.4 d	7.79 s	7.80 s
1′-NMe			2.93 s
2′			2.40 q (7.4)
2′-Me			1.25 d (7.4)
4′			3.50 m
4′-OMe			3.37 s
5′			1.94 m
6′			1.94 m
6′-Me			1.00 d (6.8)
7′			5.23 dd (5.9, 7.7)
7′-OAc			2.07 s
8′			5.56 dd (7.7, 14.9)
9′			6.25 dd (10.0, 14.9)
10′			6.15 dd (10.0, 14.4)
11′			5.71 dt (6.3, 14.4)
12′			3.90 br dd (5.4, 6.3)
16′			4.40 d (6.2), 4.75 d (6.2)

Solvent: $CDCl_3$. The coupling constants (Hz) are in parentheses.

Fig. 6. Partial structures **A** and **B** of **1**.



coupling of C-10 (δ 29.0)/12-H (δ 6.80), C-11 (δ 150.7)/10-H (δ 3.51), and C-13 (δ 150.4)/12-H confirmed the connection of partial structures **A** and **B** at C-10 and C-11 (Fig. 6).

The geometrical isomerism of the conjugated triene moiety was assumed to be 6*Z*,8*E* from the coupling constants ($J_{6,7}=11.5$ Hz, $J_{8,9}=15.0$ Hz), and 4*Z* according to the ^{13}C chemical shift of 4-CH_3 (δ 19.4).[3] Based on the above spectral analyses, the structure of **1** was proposed as shown in Fig. 1.

A comparison of this structure with known compounds from microbial origin revealed that **1** comprised a partial structure of oxazolomycin (**2**), an antitumor compound from a streptomycete.[4] The structure of **2** was elucidated, together with the stereochemistry, by ozonolysis and X-ray crystallography of the degradation products. A comparison of NMR data for **1** and diacetyloxazolomycin (**3**) showed good coincidence with respect to the corresponding carbons and protons (Table 2). The configuration at C-3 of **2** was reported to be *R*.[4]

A patent description on an herbicide, $CL_{22}T$[5], structurally identical with **1**, appeared almost at the same time as did **1** (in the name of antibiotic OM-5714[6]). Inthomycins[7], reported recently, are **1** and its stereoisomers with respect to C-4 and C-6 positions.

Experimental

UV spectrum was recorded on a Shimadzu spectrophotometer, model UV 240, and IR spectrum on a Jasco A-102 spectrophotometer. Mass spectra were obtained on JEOL mass spectrometers, model JMS D-100, DX-300, and DX-3100. NMR spectra were recorded on a Varian XL-400 NMR spectrometer with ^1H NMR at 400 MHz and ^{13}C NMR at 100 MHz.

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