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Structure Elucidation of Sesquiterpenoid from Pathogenic Fungus *Bipolaris cynodontis*

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식물 병원균 *Bipolaris cynodontis*로부터 분리한 세스퀴테르펜류 화합물의 구조 분석

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Abstract : A phytotoxic compound was isolated from a culture of *Bipolaris cynodontis*, a fungus pathogenic to Bermuda grass. The structure was determined by spectroscopic analyses including 2D NMR experiments, to be sesquiterpene having a 9-carbon unit side chain. The compound inhibits the root growth of the seedlings of Italian ryegrass and rice plant, the host plant of the *B. cynodontis*, by about 100% at 100ppm, and it is suggested that this may play an important role in the expression of the disease symptom.

요약 : *Bipolaris cynodontis*의 배양 추출물로부터 이탈리안 라이그라스 뿌리의 생육저해 활성을 나타내는 물질을 분리 정제한 다음에 2차원 NMR을 포함한 각종 기기분석을 이용하여 구조를 결정하였다. 이 화합물의 분자식은 $C_{24}H_{30}O_5$이었으며 세스퀴테르펜류 화합물로 이탈리안 라이그라스 및 벼와 같은 벼과식물에 대해서 100ppm에서 100%의 활성을 나타내는 것으로 보아 병원균의 병징 발현에 관여하는 것으로 보여진다.

Key words : phytotoxin, *Bipolaris cynodontis*, Bermuda grass, Italian ryegrass, sesquiterpene, KM-01

1. Introduction

Although the vast majority of well described phytopathogenic microorganisms are parasitic on crop plants, weeds are also attacked by the various fungi, bacteria, and viruses. There is considerable current interest in the use of plant pathogenic microbes as agents for the biological control of certain economically important weeds.[1] One potential approach to control weeds is to use phytotoxins or their derivatives for direct application to the noxious plant. Alternatively, a study of the chemistry of phytotoxins may provide useful structural information for the synthesis of novel herbicides. A necessary preludes to these approaches are the isolation, characterization and biological testing of po-

tentially phytotoxic metabolites.

Fungal pathogens which cause leaf-blight from genera such as *Helminthosporium*, *Bipolaris* and *Alternaria* are known to produce a variety of interesting phytotoxins that play important roles in the expression of disease symptoms.[2~4] In the course of my investigations into secondary metabolites of fungal origin, the author found that a culture of *Bipolaris cynodontis*, a fungal pathogen on Bermuda grass, produced a metabolite having a rather unusual structure. In this paper, the author report the result of searching for the phytotoxic compound(s) in the culture of *B. cynodontis*, together with the isolation and structural elucidation of the detected compounds. The plant toxicity of the compound was evaluated by the inhibitory activites against the root growth of the Italian ryegrass, taking the sensitivity and the convenience into account.

2. Experimental

Fungus & Plant materials

A culture of *B. cynodontis* was kindly given by Dr. M. Tsuda of Pesticide Research Institute at Kyoto University. Italian ryegrass(*Lolium multiflorum* Lam.) was used for the bioassay, the seeds of which were purchased from Takii seed Co(Kyoto, Japan).

Bioassay Methods

A specified amount of the test compound dissolved in MeOH was applied to a petri dish (35mm in diameter) in which two layers of filter paper were laid. After the solvent was evaporated, 1ml of water containing Tween 20(Nakarai Chemicals Ltd. , Kyoto, Japan) was added to this dish to dissolve the test compound. Nine germinated seedling(ca 5mm) of plant were placed in the dish and grown in the dark at 26℃ for 72 hours. The inhibitory activity was evaluated by comparing the root length to that of the controled.

Isolation of toxin

The fungus was cultured on the potato-sucrose agar plates(1000 plates, 90mm in diameter) in the dark at 26℃ for 14 days. The culture plates were then macerated in acetone, and the extracted acetone solution was concentrated at below 40℃ to an aqueous residue, which was extracted with a mixture of n-hexane, ethyl acetate and toluene(1 : 1 : 1, v/v/v). The phytotoxic components in the organic layer, which were assayed by using Italian ryegrass(*Lolium multiflorum* Lam.) as the test plant, were purified by silica gel column chromatography using stepwise elution of ethyl acetate in n-hexane. The active fraction eluted by 80% ethyl acetate in n-hexane was further purified by reversed-phase HPLC with a Cosmosil ODS-18 column(20 i.d.×250mm) and solvent system of 80% aq. MeOH.

3. Results and Discussion

The extractant from the plate-cultured *B. cynodontis* was subjected to fractionation by silica gel column chromatography, guided by the bioassay using Italian ryegrass as the test plant. An active component was detected in the fraction eluted with 80% ethyl acetate in n-hexane, which was further purified by HPLC. The phytotoxic compound **1** was eluted with the retention time of 41.2 min. under the HPLC conditions described above, and the yield was 31.4 mg.

Physicochemical data for **1** are as follows : EI-MS m/z(%) : 398(M^+, 5.3), 298(0.7), 261(0.3), 249 (0.9), 216(0.6), 146(15), 137(100), 109(58), 81(17), 55(6) : IR($CHCl_3$) Vmax, 3553, 1720, 1694, 1662 and 1636cm^{-1} : $[\alpha]D^{22}$+594. 2°(c 0.48, MeOH) : UV (MeOH) λmax nm(ε) 250 (8640)

The ^1H and ^{13}C-NMR data are listed in *Table* 1. The molecular formula of **1** was determined to be $C_{24}H_{30}O_5$ by HR-EI-MS (M^+, obsd. m/z 398.2098, calcd. 398.2093). Nine partial structures [A] to [I]

Table 1. NMR(^{1}H, 400MHz : ^{13}C, 100MHz) data for compound **1** in $CDCl_3$

Position	δH	δC
1	6.40(d, 9.8)[a]	131.2d[b]
2	6.28(dd, 9.8, 5.0)	133.3d
3	5.43(t, 5.0)	68.9d
4	1.99(m)	41.0d
5	–	36.3s
6	2.09(d, 14.4), 1.99(d, 14.4)	44.4t
7	–	74.9s
8	–	197.1s
9	6.00(s)	124.0d
10	–	161.9s
11	–	154.7s
12	6.30(s), 6.84(s)	135.7t
13	9.52(s)	192.7d
14	1.51(s)	22.6q
15	1.02(d, 6.9)	10.3q
1′	–	166.8s
2′	5.81(d, 15.4)	118.8d
3′	7.24(dd, 15.4, 10.8)	146.0d
4′	6.15(dd, 15.2, 10.8)	126.7d
5′	6.02(dd, 15.2, 7.7)	150.8d
6′	2.17(m)	38.9d
7′	1.36(m)	29.3t
8′	0.87(t, 7.5)	11.7q
9′	1.03(d, 6.9)	19.5q

[a]Proton signal multiplicity and coupling constant (J=Hz) are in parentheses

[b]All assignments are based on the results of ^{13}C-^{1}H COSY and INEPT.

were deduced as shown in *Fig.* 1, on the basis of the extensive analyses of ^{1}H(400 MHz, *Fig.* 2) and ^{13}C NMR(100 MHz, *Fig.* 3) spectra with the aid of ^{1}H-^{1}H and ^{13}C-^{1}H COSY experiments.

The connectivity of these partial structures was deduced by long-range ^{13}C-^{1}H correlations detected through an HMBC experiment, as shown in *Fig.* 4. The correlations were observed for the signals between C-1' carbonyl (δC 166.8) and the protons H-2', H-3' and H-3 : between C-5 quaternary carbon (δC 36.3) and the protons H-1, H-3, H-6, and H-15 : between C-7 quaternary carbon (δC 74.9) and

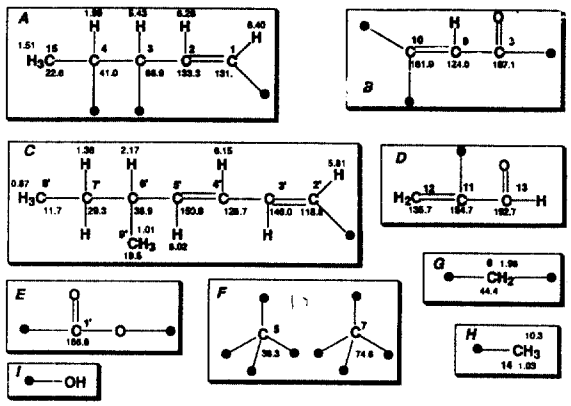

Fig. 1. Partial structures of compound **1**. The partial structures were confirmed by ^{1}H-^{1}H and ^{13}C-^{1}H COSY spectra.

protons H-6, H-9, H-12, and H-13 ; between C-8 carbonyl carbon (δC 197.1) and protons H-6 ; and between C-10 sp^2 carbon (δC 161.9) and protons H-1, H-2, H-6, H-15.

The relative stereochemistry of **1** was proposed on the basis of the ^{1}H-NMR coupling constants and NOESY spectral data. Both C2'=C3' and C4'=C5' were presumed to be trans from the relatively large coupling constants of J 2,3=15.2Hz and J 4,5=15.5Hz, respectively. The methyl groups of C-14 and C-15 were considered to be *cis* relation on the cremophilane ring, because cross peak between these methyl protons was observed in the NOESY spectrum. In addition, the substituent at C-3 was found to be in a cis relation to the C-15 methyl group, since correlations were observed between protons H-3 and H-4.

The configuration of the other parts as well as the absolute structure remained unclear. However, by comparing the physicochemical data including optical rotation, **1** was identical with a compound named KM-01[5], isolated from *Drechslera avenae* as a brassinolide inhibitor[6~9]. The absolute structure of KM-01 was determined as 3S, 4R, 5R, 7R, 6'S. The structure of **1** was related to bipolaroxin[4], a phytotoxic sesquiterpene generally-called as

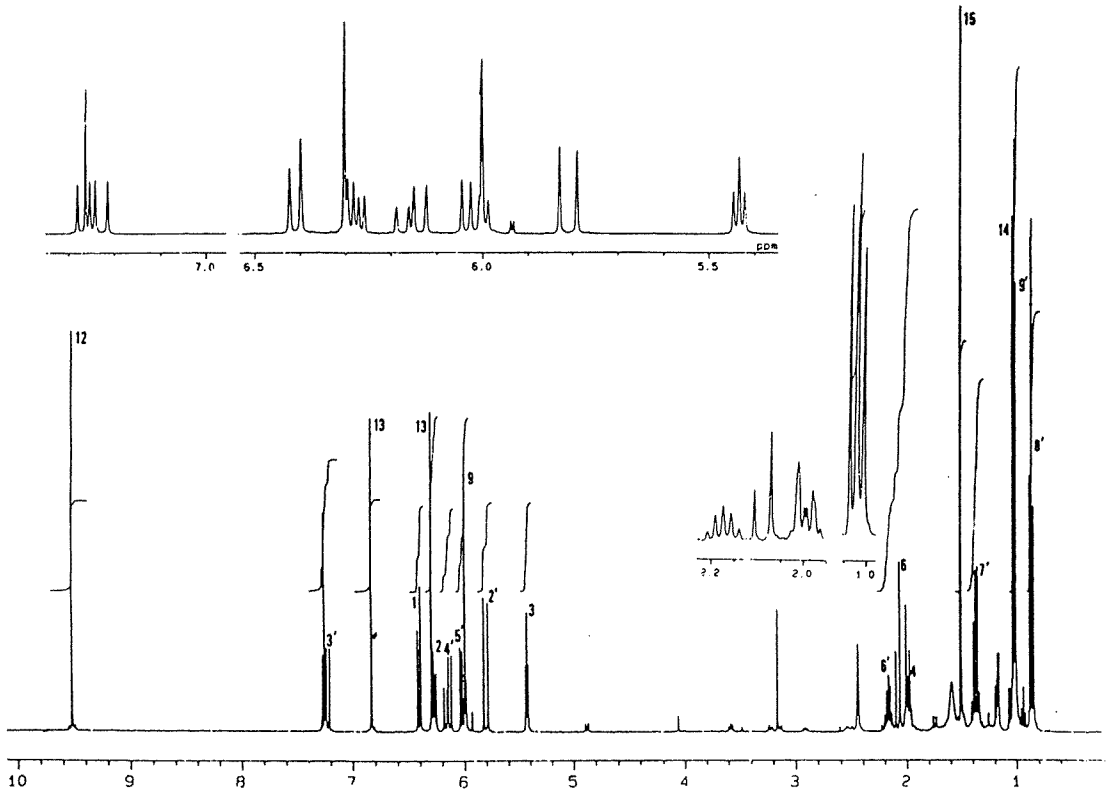

Fig. 2. ^{1}H-NMR spectrum of compound **1** (400MHz, $CDCl_3$).

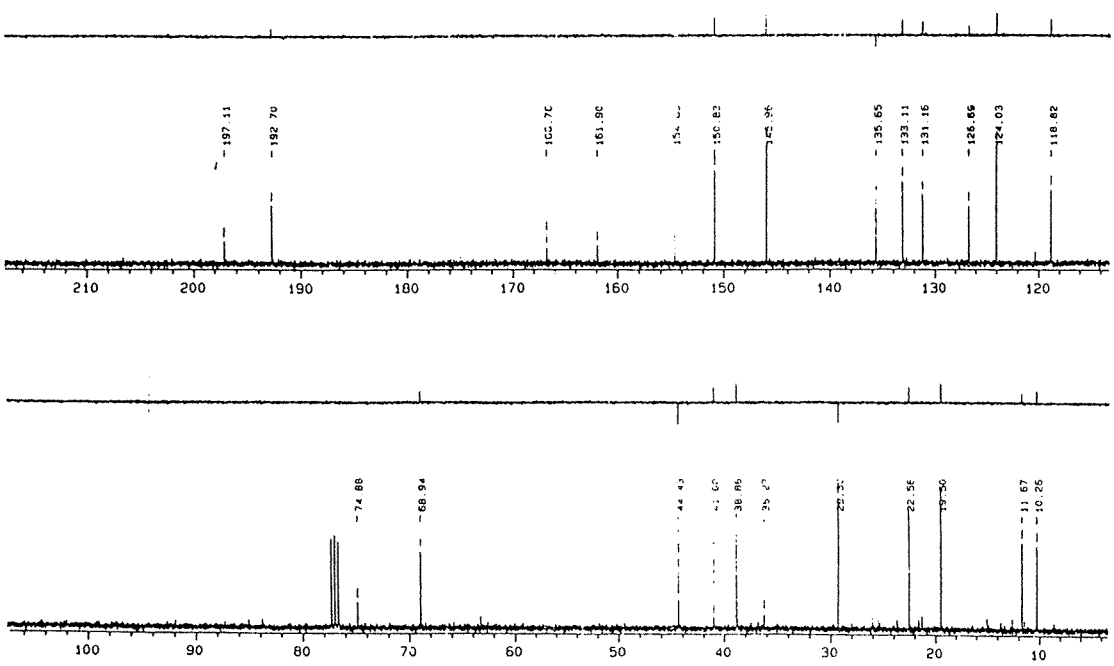

Fig. 3. ^{13}C-NMR spectrum of compound **1**(100MHz, $CDCl_3$).

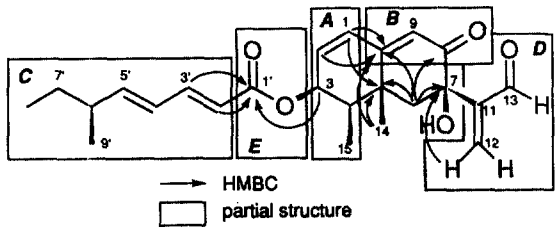

Fig. 4. Structure of compound **1**., Arrows indicate significant long-range ^{13}C-^{1}H correlations.

cremophilane type, which was isolated from the same fungal species as that used in this study.

Compound **1** inhibited the root growth of the seedlings of Italian ryegrass and rice plant, the host plant of the *B. cynodontis*, by about 100% at 100ppm. These results suggest that compound **1** may play an important role in the expression of the disease symptoms.

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