

Structure Elucidation of New Cochlioquinol Derivatives from Pathogenic Fungus *Bipolaris cynodontis*

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식물 병원균 *Bipolaris cynodontis*로부터 분리한 새로운 Cochlioquinol 유도체의 구조 분석

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Abstract : Three active compounds were isolated from the culture of a plant pathogenic fungus, *Bipolaris cynodontis*. The structure elucidation of these compounds was accomplished by 2D NMR techniques, such as ^1H - ^1H and ^{13}C - ^1H COSY, COLOC, HMBC and rotating frame NOE(ROESY). Compounds were found as derivatives of cochlioquinone and cochlioquinol that were previously isolated as phytotoxins from *B. bicolor* and *B. cynodontis*, respectively. The compounds showed phytotoxicity against Italian ryegrass, one of the host plants of *B. cynodontis*.

요약 : 이탈리아 라이그라스(*Lolium multiflorum* Lam.) 및 버뮤다그라스와 같은 벼과 잡초에 병해를 일으키는 *Bipolaris cynodontis*의 배양 추출물로부터 이탈리아 라이그라스 뿌리의 생육저해를 나타내는 3종류의 활성물질을 분리 정제하고 ^1H , ^{13}C 및 2차원 NMR을 포함한 각종 기기분석방법을 통하여 이들 화합물들의 구조를 결정하였다. 이들 화합물들은 저자에 의하여 보고된 바 있는 cochlioquinol이라 명명된 화합물의 유도체들로서 지금까지 보고된 바 없는 새로운 물질이며 숙주식물 중의 하나인 이탈리아 라이그라스에 대하여 활성(20~70%)을 나타내는 것으로 보아 병원균의 병징 발현에 관여하는 것으로 보여진다.

Key words : phytotoxin, *Bipolaris cynodontis*, Bermuda grass, Italian ryegrass, cochlioquinol, cochlioquinone.

1. Introduction

Bipolaris cynodontis is a fungus originally isolated from Bermuda grass, and found to be pathogenic against various kinds of gramineous plants, causing

leaf blight. Bermuda grass is a notorious weed in the grass family¹. At more than 80 countries, it has been reported that it has a weed problem in at least 40 different crops. Therefore the phytotoxin (s) elaborated by the pathogenic fungus *B.*

cynodontis is of interest not only by its pathological significance, but also as a lead compound that may give some clue for developing a selective weed control agent 2-4. Recently author reported a new phytotoxic metabolite (1)⁵ produced by this fungus, which has a rather unique chemical structure and was named cochlioquinol. In continuing the investigation on the secondary metabolites of this fungus, the author have found additional three metabolites (2-4) that are structurally-related to the previous compound. This paper deals with the isolation and structural elucidation of these compounds.

2. Experimental

Spectral measurements

Optical rotations were measured on a JASCO model J-5 in MeOH solution at 22°C. The IR spectra were determined on a HORITA FT-200 (CHCl₃ solution). Electron ionization mass spectra (EI-MS) were obtained on a Hitachi M-80A. The UV spectra were recorded on a Simadzu UV-3000. The ¹H and ¹³C NMR spectra were determined on a Bruker AC 500(500 MHz ¹H and 125 MHz for ¹³C) and JEOL JMS-400 in CDCl₃, using the tetramethylsilane (TMS) as an internal standard. High performance liquid chromatography (HPLC) separations were performed on Hitachi L-6200 equipped with L-4200H UV-Vis detector, using a Cosmosil octadecyl silica (ODS) column (20 mm × 250 mm i. d.).

Isolation of Toxins

The fungus was cultured on potato-sucrose agar plates (1000 plates, 90mm in diameter) in the dark at 26°C for 14 days. The active principle produced by the fungus was isolated as shown in Fig. 1. The culture plates were macerated in acetone (10 liters), and the extracted acetone solution was concentrated to an aqueous residue below 40°C, that was extracted with a mixture of n-hexane,

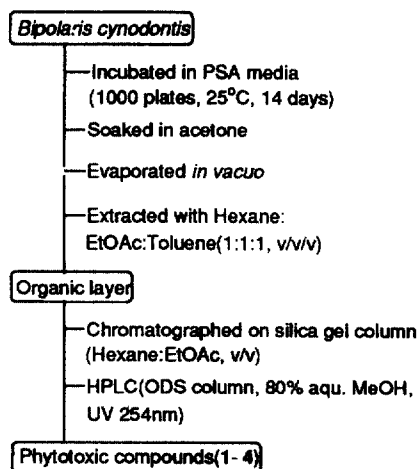


Fig. 1. Isolation of toxins from cultures of *Bipolaris cynodontis*

ethyl acetate and toluene (1 : 1 : 1, v / v / v).

The phytotoxic components(18.8g) in the organic layer, which were assayed by using Italian ryegrass(*Lolium multiflorum* Lam.) as the test plant, were purified by silica gel(1000g) 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 C18 column and solvent system of 80% aq. MeOH (flow rate, 4ml/min) to give compounds 2 (60mg, rt 30.4 min), 3 (17.2mg, rt 48.9min) and 4 (24.4mg, rt 44.4 min).

Physicochemical data

2 : EI-MS m/z(%) : 548(M⁺, 1), 530 (4), 512 (6), 490 (2), 457 (8), 431 (35), 404 (7), 371 (38), 345 (10), 327 (37), 307 (17), 283 (7), 249 (12), 203 (22), 180 (42), 165 (59), 121 (45), 97 (25), 81 (22), 59 (82), 43 (100), [α]_D²²+236.2°(c=1.84, MeOH) : UV (MeOH) λ_{max} nm (ϵ) 252 (7761), 300 (4776)

3 : EI-MS m/z(%) : 560(M-18, 3.6), 546 (2.4), 529 (2.4), 529 (100), 512 (23), 497 (24), 469 (19), 451 (8), 369 (5), 305 (14), 263 (9), 247 (10), 191 (12), 165 (14), 143 (28), 97 (22), 71 (13), 59 (77) : IR ν_{max} (KBr) 3480, 2980, 1735, 1620 cm⁻¹, [α]_D²²

Table 1. ¹H-NMR spectral data in CDCl₃ for compounds 1-4

position	1	2	3	4
1	0.88(t)	0.95(t)	0.88(t)	0.87(t)
2	1.34(m)	1.35(m)	1.07, 1.40	1.09, 1.40
3	2.82	1.60	1.61	1.57
4	—	3.47(dd, 8.3, 1.7) ^a	3.98(t, 4.9)	4.05(t, 4.9)
5	3.78(d, 7.07)	2.71	2.57(m)	2.62(m)
11	6.03(s)	6.13(s)	—	—
12	4.86(d, 10.5)	4.87(d, 10.5)	4.74(d, 10.3)	4.76(d, 10.3)
13	1.70(d, 10.5)	1.70(d, 10.5)	1.65(d, 10.3)	1.65(d, 10.3)
15	1.88	1.91	1.85, 2.02	1.85, 2.10
16	1.50, 1.75	1.50, 1.75	1.51, 1.77	1.51, 1.78
17	3.15(dd, 12.0, 3.7)	3.14(dd, 12.0, 3.7)	3.15(dd, 11.9, 3.7)	3.17(dd, 11.9, 3.7)
19	1.40, 2.51	1.41, 2.50	1.42, 2.50	1.40, 2.46
20	1.37, 1.60	1.49, 1.56	1.42, 1.60	1.40, 1.60
21	3.24(dd, 11.7, 2.8)	3.22(dd, 11.9, 2.7)	3.22(dd, 12.0, 2.5)	3.22(dd, 12.2, 2.6)
23	1.17(s)	1.16(s)	1.14(s)	1.16(s)
24	1.18(s)	1.16(s)	1.16(s)	1.16(s)
25	1.0(s)	0.99(s)	0.97(s)	0.98(s)
26	1.23(s)	1.19(s)	1.27(s)	1.28(s)
27	1.27(d, 7.0)	1.04(d, 7.0)	1.26(d)	1.37(d, 7.3)
28	1.03(d, 6.8)	0.87(d, 6.6)	0.85(d, 6.7)	0.86(d, 6.1)
29	2.84(d, 17.2)	2.84(d, 16.3)	3.33(d, 16.2)	3.38(s)
	3.01(d, 17.2)	3.03(d, 16.3)	3.41(d, 16.2)	
31	2.17(s)	2.18(s)	2.23(s)	2.25(s)
32	—	—	3.28(s)	—

^aProton signal multiplicity and coupling constant ($J=Hz$) are in parentheses, the characters denote s, single ; d, doublet ; t, triplet ; m, multiplet

+240.4° ($c=0.09$, MeOH) ; UV (MeOH) λ_{max} nm (ϵ) 247 (10247), 314 (3457)

4 : EI-MS m/z (%) : 546 (M-18, 2), 529 (2.3), 512 (7.6), 497 (1), 306 (14), 263 (7), 177 (7), 165 (23), 121 (17), 97 (15), 81 (12), 59 (100)

The ¹H and ¹³C-NMR data are listed in the Table 1 and 2, respectively.

3. Results and Discussion

Since the spectroscopic data for compounds 2-4 were generally similar to those for 1, the structures of these compounds were considered to be closely related each other. Thus structure elucidation of each compound was carried out on the basis of the

comparison of the spectral data as follows.

Compound 2 : The molecular formula of 2 was established as C₃₁H₄₈O₈ by HR-EI-MS (M⁺, obsd. m/z 548.3340, calcd. 548.3350) in combination with ¹H and ¹³C NMR data. The ¹³C NMR spectrum was similar to that of 1 except for one signal : compound 2 had an sp³ carbon presumably connected to oxygen atom (δC at 80.8 ppm), while a ketone carbon signal (δC at 212.4 ppm) was observed in 1. This sp³ carbon signal was assigned to C-4, because the proton connected to this carbon was correlated to H-3 and H-5 in the ¹H-¹H COSY. The 4-substituted-2,5-cyclohexadienone moiety in 2, constructed on the basis of COLOC spectrum, was the.

Table 2. ^{13}C -NMR spectral data in CDCl_3 for compounds 1-4

position	1	2	3	4
1	11.5q*	11.1q	11.7q	11.7q
2	26.4t	27.1t	24.8t	24.8t
3	46.4d	36.5d	38.9d	39.0d
4	212.4s	80.8d	90.7d	90.3d
5	44.8d	35.9d	37.4d	37.4d
6	155.5d	162.1s	156.4s	157.9s
7	70.9s	69.6s	97.8s	94.1s
8	166.2s	167.4s	161.2s	163.5s
9	112.8s	112.6s	113.4s	110.3s
10	186.4s	187.5s	188.2s	188.1s
11	127.1d	125.5d	126.0s	124.4s
12	63.2d	63.4d	63.2d	63.1d
13	51.6d	51.8d	52.5d	52.1d
14	82.7s	82.7s	82.3s	82.5s
15	37.7t	37.7t	37.6t	37.5t
16	25.1t	25.3t	25.3t	25.3t
17	83.8d	83.9d	83.8d	83.8d
18	36.7s	36.7s	36.7s	36.7s
19	38.5t	38.6t	38.4t	38.4t
20	21.5t	21.6t	21.6t	21.6t
21	85.2d	85.1d	85.1d	85.1d
22	72.0s	72.0s	71.9s	71.9s
23	23.6q	23.6q	23.7q	23.7q
24	25.9q	26.0q	26.0q	26.0q
25	12.5q	12.6q	12.6q	12.6q
26	21.2q	21.2q	21.2q	21.4q
27	17.3q	17.0q	19.0q	19.4q
28	16.1q	12.0q	14.2q	14.2q
29	47.9t	50.7t	40.3t	40.1t
30	206.1s	204.8s	204.5s	205.1s
31	31.4q	31.3q	30.0q	30.0q
32	—	—	51.5q	—

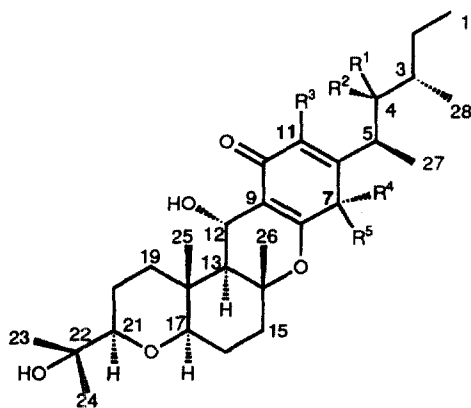
*All assignments are based on the results of ^{13}C - ^1H COSY and INEPT.

The characters s,d,t and q denote methyl, methylene, methine and quaternary carbon respectively.

same as that of 1. The $-\text{CH}_2-\text{CO}-\text{CH}_3$ moiety was connected to C-7 by the following correlations : between the protons H-11 and C-5, C-7, and C-9 ; between H-29 methylene proton signals and the carbons C-5, C-7 and C-8 ; between C-30 and protons H-29, H-31. Based on these results and the molecular formula of 2, lacking two hydrogens from 1, 2 was determined to be reduced form of 1 at C-4

carbonyl group. The stereochemistry relative to the methyl groups C-25 and C-26 was inferred from the ROESY experiment.

Compound 3 : The molecular formula of 3 was established as $\text{C}_{32}\text{H}_{50}\text{O}_9$ by elemental analysis(found : C, 66.53 ; H, 8.66 ; O, 24.81%. calcd. C, 66.41 ; H, 8.71 ; O, 24.88%) in combination with the NMR.



- 1; $R_1=R_2=O, R_3=H, R_4=OH$ & $R_5=CH_3C(=O)CH_2-$
 2; $R_1=R_3=H, R_2=R_4=OH$ & $R_5=CH_3C(=O)CH_2-$
 3; $R_1=H, R_2=R_4=OH, R_3=CH_3C(=O)CH_2-$ & $R_5=CH_3O$
 4; $R_1=H, R_2=R_4=R_5=OH$ & $R_3=CH_3C(=O)CH_2-$

Fig. 2. Structures of compound 1-4

spectra. The 1H NMR and ^{13}C NMR spectra for 3 were similar to those of 1 and 2. However, the singlet of olefin proton (δ H 6.5 ppm) corresponding to H-11 of 1 or 2 was not observed for 3, indicating that C-11 has a substituent other than hydrogen atom. The COLOC experiments revealed that the methylene protons of $-CH_2-CO-CH_3$ moiety which is commonly present in compounds 1-4, were not correlated to C-7 and C-8 but to C-6, C-10 and C-11. Therefore this moiety was allocated at C-11. The carbon C-7 in 3 was more deshielded (δ C 97.8 ppm), compared with 1 and 2 (δ C 80.51 ppm for 1). Since the correlation between C-7 and the methoxy protons (δ H 3.28 ppm) was observed in the COLOC spectrum, the carbon C-7 was deduced to form a hemiketal substructure bearing methoxy group. From the all data obtained the structure of 3 was determined.

Compound 4: The observed 1H NMR and ^{13}C NMR spectra were very similar to those of 3 except absence of signals corresponding to the methoxy group of 3. Since the ^{13}C - 1H COSY and

HMBC experiments demonstrated that most parts of 4 were identical to those of 3, it was concluded that the methoxy group at C-7 of 3 is replaced by a hydroxyl group in 4 forming a geminal diol.

Although compounds, 2-4 were closely related to 1, each of them has never been reported yet. These compounds including 1 are derivatives of cochlioquinones⁶, which have been shown to be produced as phytotoxins by *Bipolaris bicolor*, a filamentous fungus also pathogenic to gramineous plants. Compounds 2-4 inhibited the root growth of Italian ryegrass seedlings, one of the host plants of *B. cynodontis*, by 20-70% at 100ppm. These inhibitory activities are almost as potent as that of 1, but much weaker than those of cochlioquinone derivatives. This results again indicate that the quinone structure plays an important role in the growth inhibition by those compounds.

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