Studies on the Metabolites of Mycoparasitic Fungi. I. Metabolites of *Cladobotryum varium*

Yasuhiro TEZUKA,[a] Qing HUANG,[a] Tohru KIKUCHI,*[,a] Arasuke NISHI,[b] and Keisuke TUBAKI[c]

Research Institute for Wakan-Yaku (Oriental Medicines)[a] and Faculty of Pharmaceutical Sciences,[b] Toyama Medical and Pharmaceutical University, 2630 Sugitani, Toyama 930–01, Japan and College of Pharmacy, Nihon University,[c] 7–7 Narashinodai, Funabashi-shi, Chiba 274, Japan. Received July 15, 1994; accepted August 24, 1994

Six new α-pyrone derivatives, named cladobotrins I—VI (1—3, 5, 10, 11), and a known one, rosellisin aldehyde (6), were isolated from the culture broth of a mycoparasitic fungus, *Cladobotryum varium* NEES *et* DUBY (= *Didymocladium ternatum* BON.). Their structures were established by spectroscopic methods including two-dimensional NMR techniques. Among the compounds obtained, three α-pyrone derivatives, having a formyl group at the C-5 position, showed inhibitory activity against the mycelial growth of a fungus, *Ganoderma lucidum* (FR.) KARST., the fruit bodies of which are used as the oriental crude drug "Lin-Chi".

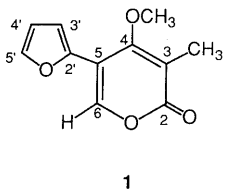
Keywords *Cladobotryum varium*; cladobotrin; α-pyrone; antifungal metabolite; mycoparasitic fungus; Hyphomycetes

The oriental crude drug "Lin-Chi" consists of the fruit bodies of the fungus *Ganoderma lucidum* (FR.) KARST. (Polyporaceae), and is used for the alleviation of minor disorders and to promote vitality and longevity.[1] Cultivation of *G. lucidum* has led to its use as a constituent of many health drinks or foods. The cultivation of *G. lucidum*, however, suffers from problems associated with certain mycoparasitic fungus which injure *G. lucidum*. We obtained one such mycoparasitic fungus, *Cladobotryum varium* NEES *et* DUBY (= *Didymocladium ternatum* BON., Hyphomycetes),[2] and found that the culture broth of this fungus showed inhibitory activity against the mycelial growth of *G. lucidum*. In the course of our search for antifungal substances, we have examined the culture of the fungus and identified eleven metabolites. In this paper, we wish to report the isolation and structure elucidation of these metabolites.

Culture broth of *C. varium* was separated into mycelia and medium by filtration and the mycelia were extracted successively with CH_2Cl_2 and MeOH to give fractions M-C and M-M, respectively. The medium was similarly treated to yield a CH_2Cl_2-soluble fraction (fraction F-C), an MeOH-soluble fraction (fraction F-M), and an MeOH-insoluble fraction (fraction F-H). Among them, only fraction F-C showed inhibitory activity against the mycelial growth of *G. lucidum*. This fraction was separated by a combination of silica gel column chromatography and preparative TLC procedures to give seven α-pyrone derivatives along with four amino acid derivatives.

The amino acid derivatives were identified as the known compounds, 2-acetyl-3*H*-quinazolin-4-one (**4**),[3] *cyclo*-(L-Pro–L-Leu) (**7**),[4] 2-pyruvoylaminobenzamide (**8**),[3,5] and *cyclo*-(L-Pro–L-Val) (**9**),[4] while one of the α-pyrone derivatives was identified as rosellisin aldehyde (**6**)[6] by spectral analysis and comparison with the literature data. The other six α-pyrone derivatives were all new compounds, and were named cladobotrins I—VI (**1**—**3**, **5**, **10**, **11**).

Cladobotrin I (**1**), a colorless amorphous solid, showed the molecular ion peak at *m/z* 206 in the MS and the molecular formula was determined to be $C_{11}H_{10}O_4$ by high-resolution MS (HR-MS). It showed a UV absorption



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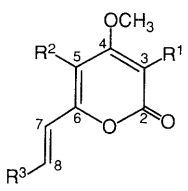
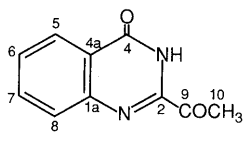


	R^1	R^2	R^3
2	CH_3	CHO	CH_3
3	CH_3	CHO	CO_2CH_3
5	CH_3	CH_2OH	CH_3
6	CH_2OH	CHO	CO_2CH_3
10	H	CH_2OH	CH_3
11	CH_3	CH_2OH	CH_2OH
12	CH_2OH	CH_2OH	CO_2CH_3



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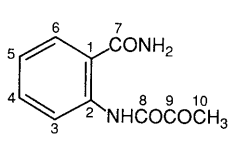

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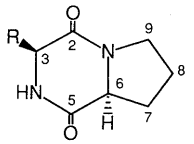


7: R = $CH_2CH(CH_3)_2$
9: R = $CH(CH_3)_2$

Chart 1

TABLE I. ^{1}H-NMR Data for **1**—**3**, **5**, **6**, **10**, and **11** (J in Hz)

Position	**1**[a]	**2**[a]	**3**[b]	**5**[a]	**6**[a]	**10**[c]	**11**[b]
3						5.61 s	
6	7.79 s						
7		7.29 dq (15.3, 1.5)	8.03 d (15.0)	6.39 dq (15.2, 1.4)	8.15 d (15.6)	6.57 dq (15.5, 1.4)	6.71 dt (15.5, 1.8)
8		7.16 dq (15.3, 7.0)	6.73 d (15.0)	6.76 dq (15.2, 7.0)	6.98 d (15.6)	6.76 dq (15.5, 7.0)	6.60 dt (15.5, 4.0)
9		2.01 dd (7.0, 1.5)		1.93 dd (7.0, 1.4)		1.95 dd (7.0, 1.4)	4.19 2H, ddd (5.2, 4.0, 1.8)
3-CH_3	2.11 s	2.08 s	2.03 s	2.06 s			1.94 s
3-CH_2OH					4.65 s		
4-OCH_3	3.82 s	3.92 s	3.94 s	3.93 s	4.20 s	3.91 s	3.86 s
5-CHO		10.09 s	10.06 s		10.18 s		
5-CH_2OH				4.50 s		4.47 s	4.30 d (5.2)
5-CH_2OH							5.03 t (5.2)
9-OCH_3			3.78 s		3.84 s		
9-OH							5.07 t (5.2)
3′	6.66 br d (3.4)						
4′	6.46 dd (3.4, 1.8)						
5′	7.42 dd (1.8, 0.9)						

a—*c*) Measured in $CDCl_3$, DMSO-d_6, and CD_3OD, respectively.

TABLE II. ^{13}C-NMR Data for **1**—**3**, **5**, **6**, **10**, and **11**

Position	**1**[a,d]	**2**[a,e]	**3**[b,d]	**5**[a,f]	**6**[a,e]	**10**[c,f]	**11**[b,d]
2	164.6 s	162.9 s	161.5 s	164.8 s	162.2 s	166.8 s	163.5 s
3	113.1 s	109.3 s	113.1 s	110.3 s	113.1 s	89.8 d	109.9 s
4	164.3 s	167.1 s	165.4 s	167.7 s	165.5 s	173.5 s	167.7 s
5	110.8 s	110.1 s	114.5 s	112.0 s	113.9 s	112.3 s	113.8 s
6	146.0 d	162.0 s	156.4 s	155.1 s	159.4 s	159.6 s	154.4 s
7		143.4 d	131.5 d	135.8 d	131.3 d	138.2 d	117.0 d
8		121.0 d	127.2 d	119.4 d	113.1 d	121.8 d	138.3 d
9		19.2 q	165.2 s	18.7 q	167.2 s	19.6 q	60.1 t
3-CH_3	10.3 q	10.0 q	10.2 q	10.6 q			10.1 q
3-CH_2OH					55.9 t		
4-OCH_3	60.4 q	61.6 q	62.0 q	63.7 q	63.5 q	58.1 q	61.2 q
5-CHO		187.6 d	188.6 d		187.0		
5-CH_2OH				55.2 t		54.4 t	52.8 t
9-OCH_3			52.2 q		55.3 q		
2′	144.9 s						
3′	109.6 d						
4′	111.6 d						
5′	142.1 d						

a—*c*) Measured in $CDCl_3$, DMSO-d_6, and CD_3OD, respectively. *d*) Assignments were based on the long-range correlations observed in HMBC or long-range ^{1}H–^{13}C COSY spectra. *e*,*f*) Assignments were based on comparison with the data for **3** and **11**, respectively.

at 260.5 nm (log ε, 4.19) and IR absorptions at 1710 (CO), 1640, and 1550 (C=C) cm^{-1}. The ^{1}H- and ^{13}C-NMR spectra of **1**, analyzed by ^{1}H–^{1}H shift correlation spectroscopy (COSY) and selective decoupling methods, revealed the presence of a methyl group and a methoxyl group (Tables I, II). In addition, they showed signals due to an sp^2 quaternary carbon (δ_C 144.9) and three coupled sp^2 methines (δ_H 7.42, dd, J=1.8, 0.9 Hz, δ_C 142.1; δ_H 6.66, br d, J=3.4 Hz, δ_C 109.6; δ_H 6.46, dd, J=3.4, 1.8 Hz, δ_C 111.6) ascribable to a 2-furyl group.[7] Moreover, the ^{13}C-NMR spectrum of **1** showed the presence of four sp^2 quaternary carbons and an sp^2 methine, suggesting that **1** is a pyrone derivative having three substituents (methyl, methoxyl, and furyl groups).

Locations of the substituents were determined by analyses of the difference nuclear Overhauser effect (NOE) and ^{1}H-detected heteronuclear multiple-bond connectivity (HMBC) spectra.[8] In the difference NOE spectra, NOE's were observed between the methyl and methoxyl protons and between the methoxyl protons and the 3′-proton of the furyl group. Thus, the methyl, methoxy, and furyl substituents should be adjacent to each other, in that order. On the other hand, in the HMBC spectrum (Fig. 1), the methyl protons at δ_H 2.11 (3-CH_3) showed long-range correlations with the quaternary carbons at δ_C 113.1 (C-3), 164.3 (C-4), and 164.6 (C-2). From a consideration of chemical shift values, the first one could be ascribed to a methyl-substituted carbon, while the latter two were considered to be a methoxy-substituted carbon and a carbonyl one. Therefore, the methyl group must be located at the C-3 position and cladobotrin I was determined to be 5-(2-furyl)-4-methoxy-3-methyl-2-pyrone (**1**). Other long-range correlations observed in the HMBC spectrum also supported this structure.

Cladobotrin III (**3**) was obtained as a colorless amorphous solid, and its molecular formula was

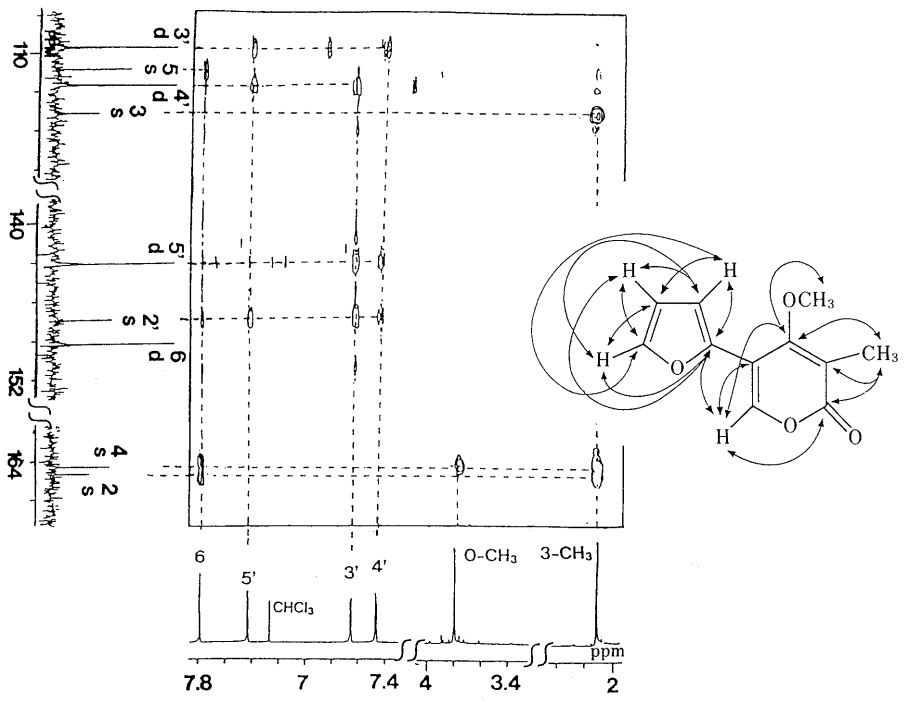


Fig. 1. HMBC Spectrum of **1** in $CDCl_3$
Sample, 12 mg; $^{lr}J_{CH}$ = 8.3 Hz; 12 h run.

determined to be $C_{12}H_{12}O_6$ (*m/z* 252) by MS and HR-MS measurements. It showed UV absorptions at 235 (log ε, 3.24) and 341 (3.01) nm and IR absorptions at 1720 and 1710 (CO) cm^{-1}, which were similar to those of rosellisin aldehyde (**6**), suggesting that **3** might be an α-pyrone derivative.[9]

The ^{1}H-NMR spectrum of **3** exhibited signals due to two methoxyls (δ_H 3.94, 3.78), a *trans*-olefin (δ_H 8.03, 6.73, J = 15.0 Hz), and a formyl group (δ_H 10.06), like that of **6** (Table I). It was, however, characterized by the appearance of a signal due to a tertiary methyl group at δ_H 2.03 instead of the signal of the hydroxymethyl group in **6** (Table I). The ^{13}C-NMR spectrum of **3** showed six quaternary sp^2 carbon signals (δ_C 165.4, 165.2, 161.5, 156.4, 114.5, 113.1) and the spectral pattern was also similar to that of **6**, except for the signal due to the methyl group (Table II).

In order to determine the structure of **3**, difference NOE, ^{1}H–^{13}C COSY, and HMBC spectra were measured. The results of a series of difference NOE experiments revealed the presence of NOE's between the methyl (δ_H 2.03) and methoxyl (δ_H 3.94) protons, between the methoxyl and formyl (δ_H 10.06) protons, and between the formyl proton and the olefinic proton at δ_H 8.03 (7-H) (Fig. 2, left). In the HMBC spectrum, the methyl protons at δ_H 2.03 (3-CH_3) showed a long-range correlation with the carbon signal ascribable to a carbonyl carbon (δ_C 161.5) (Fig. 2, right). From these observations and other long-range correlations depicted by arrows in Fig. 2, cladobotrin III was determined to be 5-formyl-4-methoxy-6-[2-(methoxycarbonyl)-*E*-ethenyl]-3-methyl-2-pyrone (**3**).

Cladobotrin II (**2**), a colorless amorphous solid, $C_{11}H_{12}O_4$, was obtained as one of the minor constituents. The UV, IR, and ^{1}H- and ^{13}C-NMR spectra of **2** were

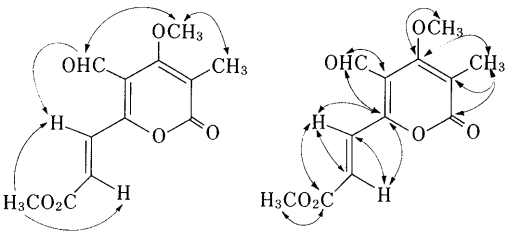
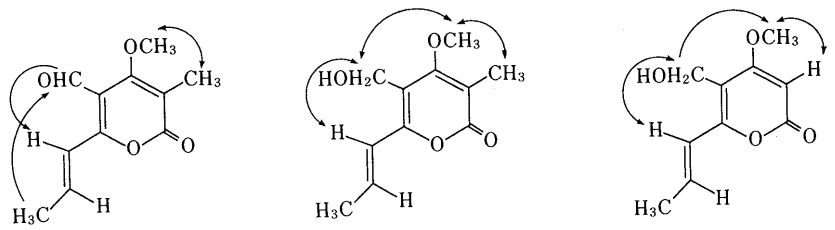


Fig. 2. NOE (Left) and Long-Range Correlations (Right) Observed in the Difference NOE and HMBC Spectra of **3**

similar to those of **3** and **6**. However, its ^{1}H- and ^{13}C-NMR spectra showed signals due to an *E*-propenyl group (δ_H 2.01, dd, J = 7.0, 1.5 Hz, δ_C 19.2, q; δ_H 7.16, dq, J = 15.3, 7.0 Hz, δ_C 121.0, d; δ_H 7.29, dq, J = 15.3, 1.5 Hz, δ_C 143.4, d) instead of the signals due to the 2-(methoxycarbonyl)-ethenyl group in **3** and **6**. From these data and the results of NOE experiments (Fig. 3), cladobotrin II was determined to be 5-formyl-4-methoxy-3-methyl-6-(*E*-propenyl)-2-pyrone (**2**).

Cladobotrin IV (**5**), a colorless amorphous solid, $C_{11}H_{14}O_4$, and cladobotrin V (**10**), colorless plates, mp 156—158 °C, $C_{10}H_{12}O_4$, were both obtained as minor constituents. Their UV, IR, and ^{1}H- and ^{13}C-NMR spectra were similar to each other and to those of **2**. The ^{1}H- and ^{13}C-NMR spectra of **5** showed signals due to a hydroxymethyl group instead of the signals due to the formyl group in **2** (Tables I, II). On the other hand, the ^{1}H-NMR spectrum of **10** showed a signal due to an isolated olefinic proton instead of the signal due to the methyl group in **5**, suggesting that **10** was a demethylated derivative of **5**. In the difference NOE experiments carried out on **5** and **10** (Fig. 3), NOE's were observed between



2 5 10

Fig. 3. NOE Correlations Observed in the Difference NOE Spectra of **2**, **5**, and **10**

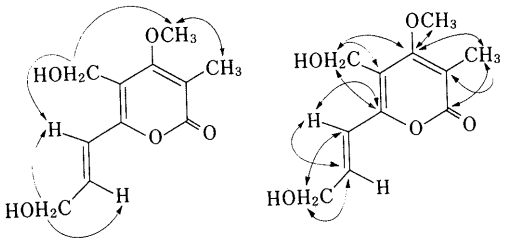


Fig. 4. NOE (Left) and Long-Range Correlations (Right) Observed in the Difference NOE and Long-Range ^{1}H–^{13}C COSY Spectra of **11**

the methoxyl and hydroxymethyl protons and between the hydroxymethyl and olefinic protons. In addition, NOE was observed between the methyl and methoxyl protons in the case of **5**, while in the case of **10**, NOE was observed between the isolated olefinic proton and the methoxyl protons. Thus, cladobotrins IV and V were determined to be 5-hydroxymethyl-4-methoxy-3-methyl-6-(*E*-propenyl)-2-pyrone (**5**) and 5-hydroxymethyl-4-methoxy-6-(*E*-propenyl)-2-pyrone (**10**), respectively.

Cladobotrin VI (**11**), a major α-pyrone derivative, was obtained as colorless plates, mp 144—146 °C, and its molecular formula was determined to be $C_{11}H_{14}O_5$ (*m/z* 226) based on elemental analysis and MS measurement. Its UV and IR spectra suggested that it was also an α-pyrone derivative.

The ^{1}H- and ^{13}C-NMR spectra of **11**, analyzed with the aid of the ^{1}H–^{1}H and ^{1}H–^{13}C COSY spectra, showed the presence of a methyl group, a methoxyl group, and a hydroxymethyl group, like those of **5** (Tables I, II). However, they showed signals due to a 3-hydroxy-*E*-propenyl group instead of the signals due to the *E*-propenyl group in **5**. From these data and the results of difference NOE experiments (Fig. 4, left), cladobotrin VI was determined to be 5-hydroxymethyl-6-(3-hydroxy-*E*-propenyl)-4-methoxy-3-methyl-2-pyrone (**11**). This structure was confirmed by the correlations observed in the long-range ^{1}H–^{13}C COSY spectrum (Fig. 4, right).

From the viewpoint of biogenesis, it is interesting that cladobotrin I (**1**), having a C4 side chain at the C-5 position, co-exists with α-pyrone derivatives having a C3 side chain at the C-6 position. From the results of biogenetic studies, Nair[6] proposed that rosellisin (**12**) and rosellisin aldehyde (**6**) are biosynthesized from a polyacetate precursor through cyclization, *C*- and *O*-methylation, and transformation of the side chain. He did not, however, indicate whether the C1 unit at the C-5 position had been introduced before cyclization or after. The fact that both **1** and other α-pyrones including **6** were obtained from the culture broth of *C. varium* suggests that they might be biosynthesized from a substituted polyacetate **14** or its congener (Chart 2).[10]

Among the compounds obtained, cladobotrins II (**2**) and III (**3**) and rosellisin aldehyde (**6**), which have a formyl group at the C-5 position, showed inhibitory activity against the mycelial growth of *G. lucidum* (Table III). This appears to indicate that the 5-formyl group is important for the inhibitory activity.

Experimental

Melting points were determined on a Yanagimoto micro melting point apparatus, and are uncorrected. Optical rotations were measured on a JASCO DIP-140 digital polarimeter at 26 °C. UV spectra were taken with a Shimadzu 202 UV spectrometer in EtOH solutions and IR spectra with a JASCO IRA-2 spectrometer in $CHCl_3$ solutions unless otherwise noted. MS and HR-MS were obtained with a JEOL JMS D-300 spectrometer (ionization voltage, 70 eV; accelerating voltage, 3 kV) using a direct inlet system. ^{1}H-, ^{13}C-, and two-dimensional (2D) NMR and difference NOE spectra were measured with a JEOL JNM-GX400 spectrometer.

Column chromatography was carried out over silica gel (Merck, Art. 7734) and the eluates were monitored by TLC. Analytical TLC and preparative TLC were carried out on precoated Merck Kieselgel 60 F_{254} plates (0.25, 0.5 mm).

Culture Medium Burkholder medium[11] was modified by replacing asparagine and biotin with casein hydrolysate (0.5%) and adjusting the pH to 5.5 with 1 N HCl. Casein hydrolysate was purchased from Wako Pure Chemical Industries, Ltd.

Culture of *Cladobotryum varium* A wild strain of *C. varium* was provided by Mr. Kayagaki and Emeritus Professor Ogita and identified by one of the authors (K. T.). Sixty Fernbach flasks (2 l), each containing 600 ml of modified Burkholder medium, were sterilized by autoclaving and inoculated with a piece of mycelial mat of *C. varium*. The culture was carried out at room temperature for 14 d under a static condition.

Assay of Inhibitory Activity against Mycelial Growth of *G. lucidum* Aliquots of 30 μl sample solutions (30—100 μg in 1 ml MeOH) were applied to three small pieces (for each concentration) of round filter paper (Toyo No. 2, 14 mm in diameter, sterilized at 160 °C for 2 h) using a micro syringe. The wet filter paper disc was then air-dried. This set of paper discs (three pieces) was put onto the surface of Yeast-malt agar medium[12] (8 ml), which had been placed in a culture dish and sterilized by autoclaving. Then a piece of mycelial mat of *G. lucidum* (1—2 mm in diameter) was placed on the surface of each filter paper disc and cultured at 26 °C for 5 d. Growth of the mycelial mat on each filter paper disc was observed with the naked eye and graded "+" (growth) or "−" (no growth) (Table III).

Preliminary Bioassay of Culture Broth Culture broth of *C. varium* (500 ml) was separated into mycelia (wet weight, 13.4 g) and medium by filtration and the mycelia were extracted successively with CH_2Cl_2 and MeOH (each 500 ml × 3) to give fractions M-C (48 mg) and M-M (245 mg), respectively. On the other hand, the medium was extracted with CH_2Cl_2 (500 ml × 4) to give a fraction F-C (152 mg). The H_2O layer was lyophilized and extracted with MeOH (500 ml × 4) to give a fraction F-M (40 mg), and the residue was lyophilized to give a fraction F-H (15 mg). Among these fractions only F-C showed inhibitory activity (30 μg/disc, − − −; 15 μg/disc, − + +; 5 μg/disc + + +), while the

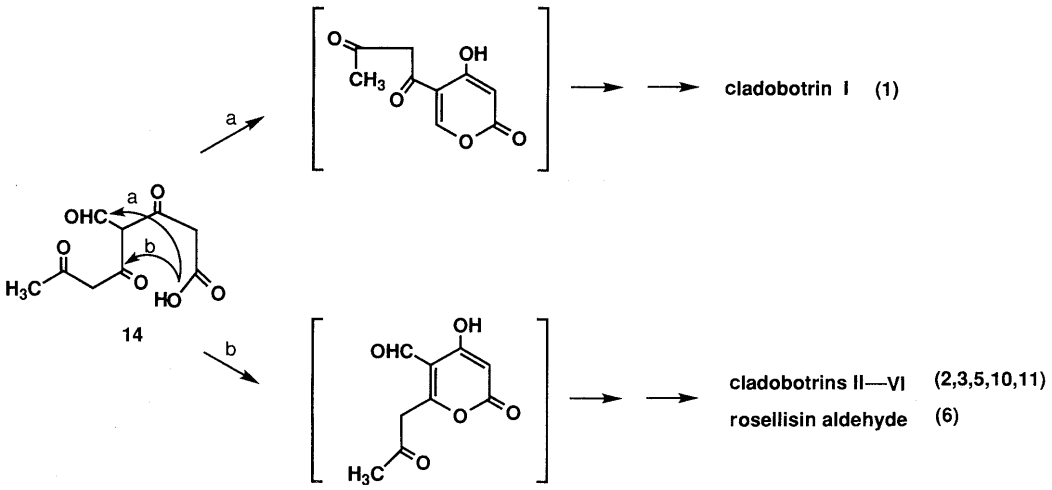


Chart 2

TABLE III. Inhibitory Effects of Compounds **1**—**11** on the Growth of Hyphae of *G. lucidum*

	Concentration (μg/disc)				
	30	15	6	3	1.5
1	+ + +				
2	− − −	− − −	− − −	− − −	− + +
3	− − −	− − −	− − −	− − −	− − +
4	+ + +				
5	+ + +				
6	− − −	− + +	+ + +		
7	+ + +				
8	+ + +				
9	+ + +				
10	+ + +				
11	+ + +				
Polyoxin	− − −	− − −	− − −	+ + +	

Each test sample was examined in triplicate: "−", no growth; "+", growth.

others showed no activity at the sample amount of 1 mg/disc.

Isolation of Metabolites The culture broth (36 l) was filtered and the filtrate was extracted with CH_2Cl_2 (35 l × 3). The CH_2Cl_2 solution was concentrated *in vacuo* and the residue (8 g) was chromatographed on a silica gel column (700 g) with $CHCl_3$ (6 l) and $CHCl_3$–MeOH gradient mixtures (100 : 1, 3 l; 100 : 3, 3 l; 100 : 10, 3 l). Eluates were collected in 15 ml portions, monitored by TLC, and then combined into a total of thirteen fractions [frs. 1—5, $CHCl_3$ eluate; frs. 6—10, $CHCl_3$–MeOH (100 : 1) eluate; frs. 11—12, $CHCl_3$–MeOH (100 : 3) eluate; fr. 13, $CHCl_3$–MeOH (100 : 10) eluate].

Fractions 2 (24 mg) and 8 (300 mg) gave cladobotrin I (**1**) and *cyclo*-(L-Pro–L-Val) (**9**), respectively.

Fraction 3 (29 mg) was further purified by preparative TLC with $CHCl_3$–CH_3CN (95 : 5) to give cladobotrin II (**2**, 9 mg). Fraction 4 (173 mg) was separated by preparative TLC with $CHCl_3$–CH_3CN (90 : 10) to yield an additional crop of **2** (3 mg) and cladobotrin III (**3**, 19 mg), and 2-acetyl-3*H*-quinazolin-4-one (**4**, 13 mg) in order of increasing polarity. Similarly, preparative TLC of fraction 5 (78 mg) with $CHCl_3$–CH_3CN (80 : 20) yielded cladobotrin IV (**5**, 27 mg).

Fraction 6 (84 mg) was also subjected to preparative TLC with $CHCl_3$–CH_3CN–acetone (70 : 20 : 10) to give rosellisin aldehyde (**6**, 6 mg) from the less polar zone and *cyclo*-(L-Pro–L-Leu) (**7**, 4 mg) from the more polar zone.

Fraction 9 (138 mg), on concentration, deposited a crystalline mass which was recrystallized from $CHCl_3$–MeOH to give 2-pyruvoylaminobenzamide (**8**, 9 mg). The mother liquor was purified by preparative TLC with $CHCl_3$–MeOH (95 : 5) to yield **9** [*cyclo*-(L-Pro–L-Val), 17 mg].

Fraction 10 (137 mg) was separated by preparative TLC with $CHCl_3$–MeOH (95 : 5) to give additional crops of **8** (2-pyruvoylaminobenzamide, 23 mg) and **9** [*cyclo*-(L-Pro–L-Val), 9 mg] and cladobotrin V (**10**, 24 mg) in order of increasing polarity.

Fraction 12 (123 mg), on concentration, afforded a crystalline mass, which was recrystallized from $CHCl_3$–MeOH to give cladobotrin VI (**11**, 71 mg).

Cladobotrin I (1) A colorless amorphous solid. UV λ_{max} nm (log ε): 260.5 (4.19). IR ν_{max} cm^{-1}: 1710, 1640, 1550. ^{1}H- and ^{13}C-NMR: Tables I and II. MS *m/z*: 206 (M^+, 100), 178 (M^+-CO, 15), 177 (M^+-CHO, 35), 163 ($M^+-CO-CH_3$, 15), 149 ($M^+-CHO-CO$, 16). HR-MS: Found 206.0611, Calcd for $C_{11}H_{10}O_4$ (M^+) 206.0579; Found 178.0625, Calcd for $C_{10}H_{10}O_3$ 178.0629; Found 177.0563, Calcd for $C_{10}H_9O_3$ 177.0551; Found 163.0383, Calcd for $C_9H_7O_3$ 163.0395; Found 149.0599, Calcd for $C_9H_9O_2$ 149.0602.

Cladobotrin II (2) A colorless amorphous solid. UV λ_{max} nm (log ε): 231 sh (4.05), 261 (3.99), 284 (3.95), 334 (3.91). IR ν_{max} cm^{-1}: 1720, 1690, 1640, 1600, 1540. ^{1}H- and ^{13}C-NMR: Tables I and II. MS *m/z*: 208 (M^+, 25), 193 (M^+-CH_3, 100), 180 (M^+-CO, 4), 165 ($M^+-CO-CH_3$, 7). HR-MS: Found 208.0745, Calcd for $C_{11}H_{12}O_4$ (M^+) 208.0736.

Cladobotrin III (3) A colorless amorphous solid. UV λ_{max} nm (log ε): 235 (3.24), 341 (3.01). IR ν_{max} cm^{-1}: 1720, 1710, 1600. ^{1}H- and ^{13}C-NMR: Tables I and II. MS *m/z*: 252 (M^+, 9), 221 (M^+-OCH_3, 52), 193 ($M^+-CO_2CH_3$, 100), 165 ($M^+-CO_2CH_3-CO$, 6). HR-MS: Found 252.0630, Calcd for $C_{12}H_{12}O_6$ (M^+) 252.0634; Found 221.0443, Calcd for $C_{11}H_9O_5$ 221.0449; Found 193.0485, Calcd for $C_{10}H_9O_4$ 193.0500.

2-Acetyl-3*H*-quinazolin-4-one (4) Colorless plates, mp 188—189 °C (acetone). UV λ_{max} nm (log ε): 229 (3.38), 303 (3.13). IR ν_{max} cm^{-1}: 3350, 1690, 1610. ^{1}H-NMR ($CDCl_3$) δ: 9.87 (1H, br s, NH), 8.36 (1H, dd, *J*=7.9, 1.5 Hz, 5-H), 7.88 (1H, dd, *J*=8.2, 1.5 Hz, 8-H), 7.85 (1H, ddd, *J*=8.2, 6.7, 1.5 Hz, 7-H), 7.64 (1H, ddd, *J*=7.9, 6.7, 1.5 Hz, 6-H), 2.77 (3H, s, 10-H_3). ^{13}C-NMR ($CDCl_3$) δ: 194.1 (s, C-9), 160.7 (s, C-4), 147.8 (s, C-1a), 145.3 (s, C-2), 134.9 (d, C-7), 129.5 (d, C-6), 129.3 (d, C-8), 127.0 (d, C-5), 123.6 (s, C-4a), 24.1 (q, C-10). MS *m/z*: 188 (M^+, 100), 160 (M^+-CO, 17), 146 (M^+-CH_2CO, 46), 119 ($M^+-CH_2CO-HCN$, 28). HR-MS: Found 188.0582, Calcd for $C_{10}H_8N_2O_2$ (M^+) 188.0586; Found 160.0612, Calcd for $C_9H_8N_2O$ 160.0636; Found 146.0496, Calcd for $C_8H_6N_2O$ 146.0480; Found 119.0391, Calcd for C_7H_5NO 119.0372.

Cladobotrin IV (5) A colorless amorphous solid. UV λ_{max} nm (log ε): 229.5 (4.46), 318 (3.97). IR ν_{max} cm^{-1}: 3380, 1690, 1645, 1605, 1550. ^{1}H- and ^{13}C-NMR: Tables I and II. MS *m/z*: 210 (M^+, 100), 195 (M^+-CH_3, 5), 182 (M^+-CO, 60), 167 ($M^+-CO-CH_3$, 17), 153 (34). HR-MS: Found 210.0897, Calcd for $C_{11}H_{14}O_4$ (M^+) 210.0892; Found 195.0657, Calcd for $C_{10}H_{11}O_4$ 195.0657; Found 182.0975, Calcd for $C_{10}H_{14}O_3$ 182.0943; Found 167.0743, Calcd for $C_9H_{11}O_3$ 167.0709.

Rosellisin Aldehyde (6) A colorless amorphous solid. UV λ_{max} nm (log ε): 233.5 (4.07), 317.5 (3.81), 328 (3.81). IR ν_{max} cm^{-1}: 3400, 1720, 1690sh, 1590. ^{1}H- and ^{13}C-NMR: Tables I and II. MS *m/z*: 268 (M^+, 0.5), 209 ($M^+-CO_2CH_3$, 100). HR-MS: Found 268.0554, Calcd for $C_{12}H_{12}O_7$ (M^+) 268.0526; Found 209.0403, Calcd for $C_{10}H_9O_5$ 209.0450.

***Cyclo*-(L-Pro–L-Leu) (7)** A colorless amorphous solid, $[\alpha]_D$ −106.8° (*c*=0.03, $CHCl_3$). IR ν_{max} cm^{-1}: 3400, 1660. ^{1}H-NMR ($CDCl_3$) δ: 5.86 (br s, NH), 4.12 (1H, t, *J*=8 Hz, 6-H), 4.02 (1H, td, *J*=10, 4 Hz, 3-H),

3.61 (1H, dt, $J=12$, 8 Hz, 9-H), 3.54 (1H, ddd, $J=12$, 8, 3.5 Hz, 9-H), 2.36 (1H, dddd, $J=13$, 8, 7, 3.5 Hz, 7-H), 2.14 (1H, m, 7-H), 2.07 (1H, m, 1′-H), 2.03 (1H, tq, $J=8$, 3.5 Hz, 8-H), 1.91 (1H, m, 8-H), 1.74 (1H, m, 2′-H), 1.52 (1H, ddd, $J=15$, 9.5, 5 Hz, 1′-H), 1.00, 0.96 (each 3H, d, $J=6.5$ Hz, 3′-H_3, 4′-H_3). ^{13}C-NMR ($CDCl_3$) δ: 170.1, 166.2 (each s, C-2, C-5), 59.0 (d, C-3), 53.4 (d, C-6), 45.6 (t, C-9), 38.7 (t, C-1′), 28.2 (t, C-7), 24.8 (d, C-2′), 22.8 (t, C-8), 23.3, 21.2 (each q, C-3′, C-4′). MS *m/z*: 210 (M^+, 0.2), 154 ($M^+ - C_4H_8$, 100), 125 (20), 70 (36). HR-MS: Found 210.1387, Calcd for $C_{11}H_{18}N_2O_2$ (M^+) 210.1385.

2-Pyruvoylaminobenzamide (8) Colorless plates, mp 181—185 °C ($CHCl_3$–MeOH). *Anal.* Calcd for $C_{10}H_{10}N_2O_3$: C, 58.25; H, 4.89; N, 13.58. Found: C, 58.22; H, 4.88; N, 13.15. UV λ_{max} nm (log ε): 247 (4.03), 303 (3.80). IR (KBr) ν_{max} cm^{-1}: 3425, 1730, 1690, 1660, 1610, 1590, 1520. ^{1}H-NMR (DMSO-d_6) δ: 12.65, 8.28, 7.73 (each 1H, br s, NH), 8.58 (1H, dd, $J=8.2$, 1.2 Hz, 3-H), 7.84 (1H, dd, $J=8.2$, 1.2 Hz, 6-H), 7.56 (1H, td, $J=8.2$, 1.2 Hz, 4-H), 7.20 (1H, td, $J=8.2$, 1.2 Hz, 5-H), 2.43 (3H, s, 10-H_3). ^{13}C-NMR (DMSO-d_6) δ: 196.3 (s, C-9), 170.2 (s, C-7), 158.6 (s, C-8), 138.0 (s, C-2), 132.2 (d, C-4), 128.7 (d, C-6), 123.3 (d, C-5), 120.6 (s, C-1), 119.8 (d, C-3), 24.1 (q, C-10). MS *m/z*: 206 (M^+, 1), 188 (24), 163 (24), 146 (100), 119 (10).

***Cyclo*-(L-Pro-L-Val) (9)** A colorless amorphous solid, $[\alpha]_D$ −72.0° ($c=0.05$, $CHCl_3$). IR ν_{max} cm^{-1}: 3400, 1660. ^{1}H-NMR ($CDCl_3$) δ: 6.07 (1H, br s, NH), 4.08 (1H, td, $J=8$, 2 Hz, 6-H), 3.93 (1H, br s, 3-H), 3.64 (1H, dt, $J=12$, 8 Hz, 9-H), 3.54 (1H, ddd, $J=12$, 9, 2 Hz, 9-H), 2.63 (1H, qqd, $J=7$, 7, 3 Hz, 1′-H), 2.37, 2.04 (each 1H, m, 7-H_2), 2.02, 1.91 (each 1H, m, 8-H_2), 1.07, 0.91 (each 3H, d, $J=7$ Hz, 2′-H_3, 3′-H_3). ^{13}C-NMR ($CDCl_3$) δ: 170.1, 165.7 (each s, C-2, C-5), 60.4 (d, C-3), 58.8 (d, C-6), 45.2 (t, C-9), 28.6 (t, C-7), 28.4 (d, C-1′), 22.4 (t, C-8), 19.2, 16.1 (each q, C-2′, C-3′). MS *m/z*: 196 (M^+, 4), 154 ($M^+ - C_3H_6$, 100), 125 (20), 70 (17). HR-MS: Found 196.1251, Calcd for $C_{10}H_{16}N_2O_2$ (M^+) 196.1212; Found 154.0755, Calcd for $C_7H_{10}N_2O_2$ 154.0743.

Cladobotrin V (10) Colorless plates, mp 156—158 °C ($CHCl_3$–MeOH). *Anal.* Calcd for $C_{10}H_{12}O_4$: C, 61.21; H, 6.17. Found: C, 60.78; H, 5.99. UV λ_{max} nm (log ε): 227 (4.38), 315.5 (4.00). IR ν_{max} cm^{-1}: 3400, 1710, 1660, 1620, 1560. ^{1}H- and ^{13}C-NMR: Tables I and II. MS *m/z*: 196 (M^+, 100), 179 ($M^+ - OH$, 9), 168 ($M^+ - CO$, 15), 154 (46), 139 (24). HR-MS: Found 196.0782, Calcd for $C_{10}H_{12}O_4$ (M^+) 196.0778.

Cladobotrin VI (11) Colorless plates, mp 144—146 °C ($CHCl_3$–MeOH). *Anal.* Calcd for $C_{11}H_{14}O_5$: C, 58.40, H, 6.24. Found: C, 58.24; H, 6.18. UV λ_{max} nm (log ε): 230 (4.02), 328 (3.73). IR (KBr) ν_{max} cm^{-1}: 3350, 1680, 1620, 1550. ^{1}H- and ^{13}C-NMR: Tables I and II. MS *m/z*: 226 (M^+, 42), 208 ($M^+ - H_2O$, 44), 197 (100), 179 (88).

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