Note

Structure of Rosellichalasin, a New Metabolite Produced by *Rosellinia necatrix*

Yasuo KIMURA, Hiromitsu NAKAJIMA
and Takashi HAMASAKI

Department of Agricultural Chemistry,
Tottori University, Koyama,
Tottori 680, Japan

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Rosellinia necatrix is a pathogenic fungus which causes white root rot to potato, soybean, tea, peanut, apple and so on.¹⁾ It is well known that this fungus produces cytochalasin E,²⁾ which has physiological activity towards mammalian cells,³⁾ microorganisms⁴⁾ and plants.^{4,5)}

We have searched in detail for new plant growth regulators in the metabolites of this fungus and succeeded in isolating of the compound designated rosellichalasin (**1**) together with cytochalasin E (**2**). This paper deals with the isolation and structural elucidation of rosellichalasin.

This fungus, *Rosellinia necatrix*, was statically cultured in a malt extract medium (40l) containing 3% of peptone at 24°C for 21 days. Dry mycelial mats were obtained by filtering the broth and repeatedly extracted with acetone. The acetone extract (5g) was purified by a silica gel column with a solvent system of benzene–acetone. The eluate with a benzene–acetone ratio of 9:1 (v/v) was evaporated, and the residual solid was crystallized from *n*-hexane–ethyl acetate to afford colorless needles of **1** (78 mg). The eluate with a benzene–acetone mixture of 4:1 (v/v) was concentrated *in vacuo*, and the residue was applied to a Sephadex LH-20 column eluted with meth-

anol. The white amorphous compound thus obtained was crystallized from *n*-hexane–acetone to give colorless needles of **2** (1.48 g).

The molecular formula of **2**, showing mp 205~206°C and $[\alpha]_D^{20} -20^\circ$ ($c=1.0$, MeOH), was determined to be $C_{28}H_{33}NO_7$ by EIMS (M^+ , m/z 495) and elementary analysis (Found: C, 67.8; H, 6.85; N, 2.59. Calcd.: C, 67.86; H, 6.71; N, 2.83%). The UV, IR, NMR and mass spectra together with $[\alpha]_D$ revealed **2** to be cytochalasin E.⁶⁾ All the proton signals of **2** were assigned by decoupling experiments and literature values^{7,8)} to be those shown in the table.

Compound **1** was recrystallized from *n*-hexane–acetone to afford colorless needles, mp 121~123°C and $[\alpha]_D^{20} -2.7^\circ$ ($c=1.0$, MeOH). The molecular formula was determined to be $C_{28}H_{33}NO_5$ by HRMS (found, 463.2343; calcd., 463.2356).

The IR absorption bands at 1665 and 1640 cm^{-1} together with the UV absorption band at 245 nm ($\epsilon=12,500$ in EtOH) indicated the presence of an α,β -unsaturated ketone.⁹⁾

Mass spectral analysis by EIMS for **1** gave a molecular ion peak at m/z 463, with fragment ion peaks at m/z 372, 190, 120 and 91 (base peak). The fragment ion peak at 372 ($M^+ -91$) is expected to have been derived from the molecular ion peak by the loss of a benzyl ion (m/z 91). That the benzyl group is part of a phenethylamine system is indicated by the presence of an $C_8H_{10}N^+$ (m/z 120) ion in the mass spectrum. The presence of intense peak at m/z 190 corresponding to $C_{11}H_{12}NO_2^+$ must contain the elements of the lactam ring and the benzyl groups.¹⁰⁾ These results together with the subsequent physicochemical properties described suggest that **1** was a cytochalasin analog.

A comparison of the ^{13}C -NMR spectral data between **1** and **2** indicates that the carbonate carbon at δ_C 149.3 (C-21) in **2** was shifted downfield to δ_C 171.8 (C-21) in **1**, which was assigned to an ester carbon. This result is well consistent with that of the IR spectrum ($\nu_{C=O}$, 1718 cm^{-1}), in which no absorption band at 1765 cm^{-1} in **2** due to a carbonate¹¹⁾ was observed in **1**.

The signal at δ_H 6.35 (1H, dd, $J=6.8, 11.0$ Hz) assignable to a vinyl proton attached to a carbon at δ_C 135.1 (C-19) exhibited couplings with the methylene protons at δ_H 2.98 (1H, dd, $J=6.8, 12.1$ Hz) and 3.23 (1H, dd, $J=11.0, 12.1$ Hz) attached to a carbon at δ_C 39.8 (C-20). Also, irradiation of the signal at δ_H 6.35 sharpened the signal at δ_H 1.83 (3H, br. s) connected to a carbon at δ_C 12.8 (C-23), indicating a long-range allylic coupling between the meth-
yl and vinyl protons.

The methyl carbon at δ_C 12.8 (C-23) indicates the connection with a C-18 carbon at δ_C 143.1 (s) in **1** due to a sp^2 carbon appearing instead of the signal at δ_C 76.7 (s, C-18) in **2**. These results prove the presence of the partial structure $-CH_2-CH=C(CH_3)-$ in **1**. The double bond was shown to be *trans*, since the coupling constant between the methyl protons at δ_H 1.83 and the proton at δ_H 6.35 was

Table. ^1H - AND ^{13}C -NMR DATA FOR **1** AND **2**, AND ^{13}C -NMR DATA FOR CYTOCHALASIN E
(H, 270 MHz; C, 68 MHz; CDCl_3)

Carbon number	Compound 1		Compound 2		Cytochalasin E ¹⁴
	δ_{C}	δ_{H}	δ_{C}	δ_{H}	δ_{C}^a
11	12.4q	1.00 d (7.3) ^a	13.1q	1.09 d (7.3)	13.1q
22	17.4q	1.13 d (6.8)	19.6q	1.16 d (6.3)	19.6q
12	19.6q	1.23 s	20.0q	1.26 s	20.1q
23	12.8q	1.83 br.s	24.3q	1.48 s	24.4q
5	36.0d	2.23 dq (5.4, 7.3)	35.8d	2.28 dq (4.9, 7.3)	35.9d
15	36.6t	2.03 m	39.1t	2.15 ddd (2.2, 3.8, 12.7)	39.2t
		2.24 m		2.67 m	
16	39.8d	3.27 m	40.8d	2.93 m	40.8d
10	44.1t	2.85 d (2H, 5.4)	44.7t	2.70 dd (6.8, 13.1)	44.2t
				2.90 dd (4.4, 13.1)	
4	48.8d	2.72 dd (2.0, 5.4)	47.8d	3.03 dd (2.9, 4.9)	47.2d
8	47.0d	2.86 dd (5.4, 10.3)	45.8d	2.63 m	45.8d
3	53.9d	3.73 m	53.7d	3.74 m	53.7d
6	57.2s		57.3s		57.4s
7	60.3d	2.67 d (5.4)	60.6d	2.63	60.7d
18	143.1s		76.7s		76.7s
9	84.7s		87.0s		87.4s
19	135.1d	6.35 br.dd (6.8, 11.0)	120.3d	5.63 d (11.7)	120.3d
4'	125.7d	7.28 m	127.3d	7.30 m	127.2d
13	127.1d	5.83 ddd (1.5, 10.3, 15.1)	128.4d	5.89 ddd (1.6, 8.3, 16.0)	128.7d
3',5'	128.8dd	7.28 m	128.9dd	7.30 m	128.8dd
2',6'	129.6dd	7.28 m	129.6dd	7.30 m	129.9dd
14	131.8d	5.44 ddd (3.9, 11.2, 15.1)	131.5d	5.23 ddd (3.8, 11.0, 16.0)	131.4d
1'	136.6s		135.9s		135.8s
20	39.8t	2.98 dd (6.8, 12.1)	142.1d	6.48 d (11.7)	142.0d
		3.23 dd (11.0, 12.1)			
21	171.8s		149.3s		149.2s
1	170.1s		170.1s		170.7s
17	205.4s		211.7s		211.9s
NH		6.69 br		6.44 br	
OH				4.34 s	

^a Coupling constants in Hz.

0.98 Hz,¹²⁾ and no nuclear Overhauser effect between them was observed in the NOE difference spectra. The carbonyl carbon at δ_{C} 205.4 in **1** shifted upfield in comparison with that δ_{C} (211.7) in **2**, indicating the α,β -double bond effect to the carbonyl carbon in the conjugated system.¹³⁾

The chemical shifts of the remaining 23 carbons in **1**, except for 6 carbons (C-17, -18, -19, -20, -21 and -23) were approximately the same as those in **2**.

On the basis of these results, the structure of **1** has been shown to be 6, 7-epoxy-10-phenyl-5,6,16,18-tetramethyl-22-oxa-[12]-cyclochalas-13,19-diene-17,21-dione.

All signals of the ^1H - and ^{13}C -NMR spectroscopic data for **1** and **2** were assigned by literature values⁶⁻¹⁴⁾ and by $^1\text{H}\{-^1\text{H}\}$ homo-spin decoupling experiments as shown in the table.

In a bioassay using lettuce seeds, **1** at a dose of 100 mg/l inhibited the germination by 50% after 24 hr. The root and

hypocotyl growth were also inhibited by 70% and 60% at the same concentration, respectively.

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