THE ILICICOLINS, ANTIBIOTICS FROM *CYLINDROCLADIUM ILICICOLA*

Sir :

Cylindrocladium ilicicola strain MFC-870 produced a variety of new antibiotics, ilicicolins A, B, C, D, E, F, G, and H. This imperfect fungus was isolated from the dead leaf of beech (*Fagus* sp.) and identified according to BOEDIJN and REITSMA[1]. Fermentations were conducted under submerged culture conditions for 14 days at 28°C in a medium containing 3 % glucose, 2 % peptone and 0.5 % sodium chloride. The final pH was adjusted to 6.8 prior to sterilization. Eighty liters of medium were used and the culture broth was treated as follows.

Hyflo-Supercel (3 kg) was thoroughly mixed in and the suspension centrifuged. The residue was extracted with methanol (40 liters) by stirring for 2~3 hours at room temperature then allowing to stand overnight. The extract was evaporated *in vacuo* at 40~50°C, leaving an oily residue which was dissolved in ether, washed with 5 % hydrochloric acid, 5 % sodium carbonate, and water. The ethereal solution was evaporated *in vacuo* to give a residue (40.2 g) which was dissolved in benzene and chromatographed on silica gel to give ilicicolins A~H.

Ilicicolin A was obtained as pale yellow prisms (940 mg, from light petroleum), $C_{23}H_{31}ClO_3$, m. p. 72~72.5°C, $[\alpha]_D^{25}$ +0.0°, M^+ 390, λ_{max}^{EtOH} 230 mμ (ε 20,700), 297 (14,560), and 343 (4,480), $\nu_{max}^{CHCl_3}$ 3510, 1635, 1251, 1109, and 906 cm^{-1}.

Ilicicolin B was obtained as pale yellow amorphous powder (80 mg, from light petroleum), $C_{23}H_{32}O_3$, m. p. 97~98.5°C, $[\alpha]_D^{25}$ ±0.0°, M^+ 356, λ_{max}^{EtOH} 223 mμ (sh) (ε 17,600) and 301.5 (17,880), $\nu_{max}^{CHCl_3}$ 3575, 3370, 1627, 1584, 1178, and 1000 cm^{-1}.

Ilicicolin C was obtained as pale yellow needles (580 mg), $C_{23}H_{31}ClO_4$, m. p. 134~136°C/143.5~146°C (from ether), $[\alpha]_D^{21}$ ±0.0°, M^+ 406, λ_{max}^{EtOH} 229.5 mμ (ε 18,900), 296 (12,800), and 349 (5,670), $\nu_{max}^{CHCl_3}$ 3510, 1707, 1633, 1253 and 1108 cm^{-1}.

Ilicicolin D* was obtained as pale yellow prisms (from ether) (1.1 g), $C_{23}H_{29}ClO_4$, m.p. 172.5~174°C, $[\alpha]_D^{21}$ −49.7°, M^+ 404, λ_{max}^{EtOH} 240.5 mμ (ε 42,100), 295 (14,360), and 345 (5,470), $\nu_{max}^{CHCl_3}$ 3510, 1708, 1635, 1252, 1109, and 972 cm^{-1}.

Ilicicolin E** was obtained as pale yellow prisms (950 mg), $C_{23}H_{27}ClO_4$, m.p. 163~166°C (from ether), $[\alpha]_D^{20}$ −139.2°, M^+ 402, λ_{max}^{EtOH} 237.5 mμ (ε 46,100), 295 (14,100), and 346 (5,600), $\nu_{max}^{CHCl_3}$ 3510, 1679, 1635, 1250, 1110, and 972 cm^{-1}.

Ilicicolin F was obtained as pale yellow needles (300 mg), $C_{25}H_{31}ClO_6$, m.p. 156~159°C (from ether), $[\alpha]_D^{20}$ −25.7°, M^+ 462, λ_{max}^{EtOH} 240.5 mμ (ε 40,450), 295 (13,000), and 349 (6,500), $\nu_{max}^{CHCl_3}$ 3510, 1739, 1720, 1635, 1252, 1110, 1025 and 972 cm^{-1}.

Ilicicolin G, $C_{23}H_{29}ClO_4$, a viscous oil (1.2 g), showed $[\alpha]_D^{22}$ +28.4°, M^+ 404, λ_{max}^{EtOH} 230 mμ (ε 14,100), 295 (10,600), and 346 (4,150), $\nu_{max}^{CHCl_3}$ 3510, 1712, 1635, 1253, 1109, 1015, and 976 cm^{-1}.

Ilicicolin H was obtained as yellow needles (from benzene) (7.8 g), $C_{27}H_{31}NO_4$, m.p. 144~150°C, showed $[\alpha]_D^{23}$ −17.4°, M^+ 433, λ_{max}^{EtOH} 248 mμ (ε 23,200) and 349 (5,300), $\nu_{max}^{CHCl_3}$ 3593, 3401, 1653, 1612, 1548, 1516, 1173, 969, and 835 cm^{-1}.

Table 1. Rf values of ilicicolins A~H on TLC (silica gel)

	Rf values	
	n-Hexane - acetone (3 : 1)	Benzene - ethyl acetate (7 : 1)
Ilicicolin A	0.60	0.67
Ilicicolin B	0.44	0.50
Ilicicolin C	0.37	0.44
Ilicicolin D	0.34	0.43
Ilicicolin E	0.31	0.39
Ilicicolin F	0.23	0.27
Ilicicolin G	0.35	0.27
Ilicicolin H	0.09	0.055

* Ascochlorin, $C_{23}H_{29}ClO_4$, m.p. 153~154°C (decomp.) was isolated as a new antibiotic[2] and its structure was elucidated by X-ray analysis[3]. This antibiotic was obtained in two forms (α- and β-form) by TLC on silica gel, and its NMR spectrum was distinguished from that of ilicicolin D. Ilicicolin D is therefore not identical with ascochlorin.

** Cylindrochlorin, $C_{23}H_{27}ClO_4$, m. p. 150~150.5°C was isolated[4], and shown to be not identical with ilicicolin E by IR and NMR spectra.

Table 2. Cytotoxicity of ilicicolins on HeLa cells

	Ilicicolins							
	A	B	C	D	E	F	G	H
Cytotoxicity ED_{50} (μg/ml)	0.2	0.3	0.2	0.02	0.003	0.003	1	2

Rf values of ilicicolins A~H on TLC (silica gel) are shown in Table 1.

Ilicicolins showed limited antibacterial activity, and inhibited only the growth of *Bacillus anthracis* at the concentration of 6 mcg/ml. Antiviral activity could not be determined in the agar diffusion plaque inhibition test, owing to the strong cytotoxicity of the ilicicolins.

Cytotoxicities of ilicicolins against HeLa cells in culture are shown in Table 2. Chromosomes aggregation in the mitotic cells is a characteristic feature of their cytotoxicity.

The acute toxicity (LD_{50}) of ilicicolin E in mice was determined intraperitoneally as 37.9 mg/kg. Ilicicolin A was not lethal at a dose of 100 mg/kg in mice.

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