

Communications to the editors

CYLINDROCHLORIN, A NEW
ANTIBIOTIC PRODUCED
BY *CYLINDROCLADIUM*

Sir:

In our screening studies for new antibiotics produced by fungi, cylindrochlorin, a new antibiotic, was isolated from the methanol extract of the mycelium of *Cylindrocladium* sp. The isolated cylindrochlorin shows an antiviral activity against Newcastle disease virus when assayed by agar-diffusion plaque-inhibition method.

Cylindrochlorin was first discovered in laboratory fermentations carried out in the following procedures: One hundred ml of the medium in a 500-ml flask, consisting of 5% glucose, 0.5% peptone, 0.2% yeast extract, 0.3% NaCl and 1% CaCO₃, was inoculated with *Cylindrocladium* grown on an agar slant and shake-cultured at 26.5°C for 4 days. Twenty ml of this culture was then transferred into 800 ml of the same medium in 5-liter flasks and incubated at 26.5°C for 4 days on a rotary shaker. The mycelium collected by filtering the culture was extracted overnight with 200~300 ml methanol at room temperature. Methanol extract obtained by filtering off the mycelium was distilled *in vacuo* to remove the methanol. The active principle was extracted from the water solution with ethylacetate. An oily residue was obtained after the removal of the solvent. Purification was performed by repeated chromatography on silicic acid (Mallinckrodt) columns. Active fraction could be eluted by chloroform or benzene. *Candida albicans* was used as a test organism for bioassays.

Cylindrochlorin forms pale yellow crystals of m. p. 150~150.5°C. It has a low solubility in water and hexane, but is readily soluble in most organic solvents including acetone, chloroform, ethylacetate, ether and benzene. It is soluble in methanol and ethanol. The presence of chlorine in the molecule was suggested by a positive

BELSTEIN reaction and confirmed by the mass spectrum, which shows the parent peak at m/e 402 accompanied with a characteristic p+2 peak at m/e 404, the abundance ratio being 3:1.

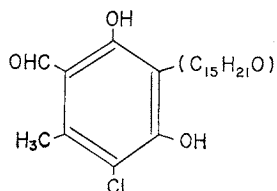
Studies of cylindrochlorin by ultraviolet, infrared and nuclear magnetic resonance spectra show a strong resemblance of the antibiotic to ascochlorin which we isolated from *Ascochyta viciae*¹⁾. Spectral differences between ascochlorin and cylindrochlorin are presented in Table 1. Cylindrochlorin differs from ascochlorin in that the molecular weight is reduced by two and that a signal in the NMR spectrum of ascochlorin at τ 8.75 is lacking in that of cylindrochlorin, while the latter possesses a new signal at τ 3.30. The UV spectra of the two antibiotics were similar to each other and the peaks in IR spectra were almost identical except those mentioned in Table 1. So the two antibiotics must have a common functional group (or groups) in their molecules. The structure of ascochlorin was recently established by X-ray crystal structure analysis²⁾. It is a derivative of salicylaldehyde. We suggest that cylindrochlorin is also a derivative of salicylaldehyde as shown in Fig. 1 and that the side moiety resembles with that of ascochlorin with the reduction of two hydrogens. But we are not yet certain of the site of reduction.

Ascochlorin was the first fungal antibiotic that possesses a salicylaldehyde moiety. It is noteworthy that a very similar metabolite has now been isolated from another species of fungi.

Table 1. Spectral differences between
ascochlorin and cylindrochlorin

	Ascochlorin	Cylindrochlorin
M.W.	404	402
U.V.	240 m μ (39,000) 290 m μ (11,000) 346 m μ (10,900)	234 m μ (49,400) 291 m μ (13,800) 347 m μ (8,500)
I.R.	1700, 1626 cm ⁻¹	1710, 1680, 1638 cm ⁻¹
N.M.R.	τ 8.75, —	—, τ 3.30

Fig. 1. Partial structure of cylindrochlorin



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References

- 1) TAMURA, G.; S. SUZUKI, A. TAKATSUKI, K. ANDO & K. ARIMA: Ascochlorin, a new antibiotic, found by paper disc agar-diffusion method. I. Isolation, biological and chemical properties of ascochlorin. (Studies on antiviral and antitumor antibiotics. I) J. Antibiotics 21 : 539~544, 1968
- 2) NAWATA, Y.; K. ANDO, G. TAMURA, K. ARIMA & Y. IITAKA: The molecular structure of ascochlorin. J. Antibiotics 22 : 511~512, 1969