TRICHOVIRIDIN AND DERMADIN FROM *TRICHODERMA* SP. TK-1

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(Received for publication October 2, 1974)

During the course of our studies on new antibiotics, we found that *Trichoderma* sp. Tk-1 produced substances A and B which showed inhibitory activities against both *Staphylococcus aureus* and *Escherichia coli*. From their chemical and physical properties, substances A and B were identified as trichoviridin[1] and dermadin[2], respectively. The strain Tk-1 was obtained from a soil sample and classified in the *Trichoderma koningii*-group according to RIFAI[3]. This paper presents some characteristics of the strain, the isolation of substances A and B, and the approach to the structure elucidation of trichoviridin.

Characteristics of the strain Tk-1 are as follows.

Morphology: Conidiophores and their branches long and slender, 10~40μ in diameter, without sterile hyphal elongation. Side branches formed in false verticils. Phialides rather slender and not crowded, nine pin-shaped, 7~15μ, disposed regularly in false whorls. Phialospores ellipsoidal, 3.2~4.0×2.4~3.2μ, smooth-walled. Globose or ellipsoidal chlamydospores, 8~9×12~13μ, produced on substrate mycelium.

Colony: Growth rapid. Aerial hyphae floccose, white or pale yellow (phialospores poor) on malt extract agar, peptone-glucose agar, CZAPEK agar, SABOURAUD agar, synthetic mucor agar, yeast-starch agar and sugar-yeast agar.

Reverse side of colony: Pale yellow on above mentioned media.

Soluble pigment: None or trace (pale yellow) in above-mentioned media.

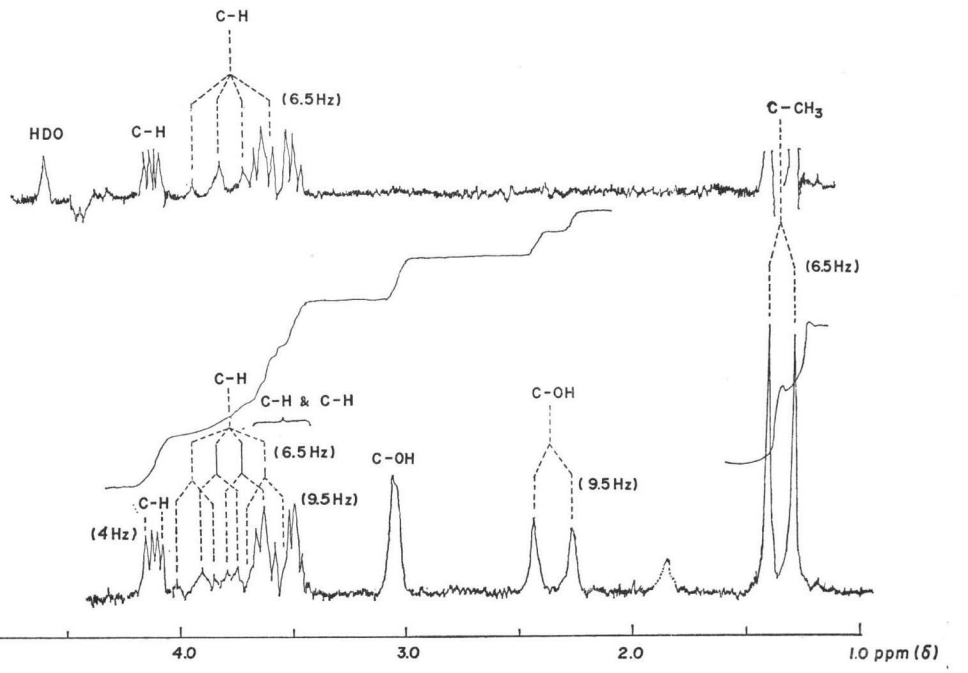
According to the classification system proposed by RIFAI[3], the strain Tk-1 may be classified into the section Aa-D-E (*Trichoderma koningii*-group, *i.e. T. koningii, T. aureoviride* and *T. harzianum*), but named species in this section are different from the strain Tk-1 in the following properties. Phialospores of *T. koningii* are mostly elliptic-subcylindrical. Colonies of *T. aureoviride* grow very slowly on malt extract agar. Phialospores of *T. harzianum* are mostly globose or subglobose. However, in other properties, including morphological and cultural properties, the strain Tk-1 shows good agreement with these species. On the other hand, *T. viride* is distinguished from this strain in the surface of phialospores, namely the former is rough-walled, but the latter smooth-walled. Therefore, the strain Tk-1 was classified as a strain of *T. koningii*-group.

The fungi were shake-cultured at 30°C for 2 days in a medium consisting of 2 % glucose, 1 % defatted soybean meal, 0.2 % NaCl and 0.05 % $CaCO_3$ (pH 6.2). The cultured broth was freed from mycelium and extracted with ethyl acetate. The ethyl acetate extract was evaporated to dryness and the residue was purified by chromatography on a column of silica gel (developing solvent: $CHCl_3$). The active fractions were concentrated to small volume and cooled. From 6 liters of cultured broth, 390 mg of crude crystals (substance A) was isolated. The crystals were further purified by direct recrystallization from chloroform. The aqueous layer was concentrated to dryness, and purified by chromatography on columns of Sephadex LH 20 (MeOH) and silica gel ($CHCl_3$). The active fractions were concentrated to small volume and cooled. From 6 liters of cultured broth, 20 mg of substance B, m.p. <85°C, was isolated. Its IR and UV spectra were superimposable on those of dermadin[2]. Therefore, substance B was identified as dermadin.

UV: λ_{max}^{EtOH} 220 nm ($E_{1cm}^{1\%}$ 84.6)
IR (KBr): 3100~2850, 2120, 1685, 1430, 1410, 1325, 975, 945, 840, 800 cm^{-1}.

The identification of substance A with trichoviridin[1] was accomplished in the following manner. Substance A, m.p. 93~95°C, $[\alpha]_D^{25}$ −36.8° (*c* 0.5, MeOH), had the molecular formula $C_8H_9NO_4$ (Anal. Calcd.: C, 52.46; H, 4.95; N, 7.65; Found: C, 52.31; H, 4.89; N, 7.65). Its IR spectrum was superimposable on that of trichoviridin[1]. However, its structure was not discussed earlier.

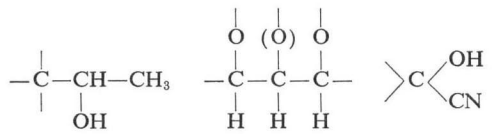
Fig. 1. NMR spectra of substance A (60 MHz, in $CDCl_3$ and $CDCl_3+D_2O$).



UV: λ_{max}^{EtOH} 215 nm ($E_{1cm}^{1\%}$ sh, 17.5)
IR (KBr): 3530, 3340, 2150, 1405, 1183, 1085, 1008, 890 cm^{-1}.
The molecular weight was unable to determine by mass spectrometry.

Trichoviridin exhibited a nitrile band at 2150 cm^{-1} in its IR spectrum and showed a positive hydroxylamine ferric chloride test[4]. On acidic treatment, trichoviridin decomposed to give some carbonyl compounds (IR : $\nu_{C=O}$ 1730 cm^{-1}) in which the nitrile bands had disappeared. These observations suggest the presence of a cyanhydrin function (C(OH)—CN).

The NMR spectra shown in Fig. 1 indicates the following partial structures:



(ABX type)

Considering with the molecular formula, these partial structures include all of the elements of trichoviridin.

Substances A and B showed inhibitory activities against *S. aureus* and *E. coli*, but both substances were very unstable on exposure to air.

Acknowledgements

The authors express deep thanks to Dr. H. NONOMURA, Faculty of Engineering, Yamanashi University, for his kind guidance and valuable advice, and to Director Dr. H. TAKAMATSU, Dr. H. NISHIMURA and Dr. K. YOKOGAWA, Research and Development Division, Dainippon Pharmaceutical Co., Ltd., for their encouragement throughout this work. Thanks are also due to the members of the Analytical Center of our Research Laboratories for micro-analyses and spectroscopic measurements.

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