A New Antifungal Substance Produced by *Myrothecium*

Sir:

Recently many strains of mold were isolated from soils by the authors and screening tests of antibiotics productivity were performed on those strains by them. As a results they have discovered a new antibiotic substance in fermentation broth of a fungus isolated from a soil sample collected in Bangkok of Thailand.

Temporarily this antibiotic was designated as A-2 material and the producing mold as strain No. 310-1. A-2 was later found to be a new member of antibiotics, the Verrucarins and Roridin group reported by Ch. Tamm *et al.*,¹⁾ i.e. Verrucarins A, B, C, D, E, F, G, H, J and Roridin A, B, C, D, E which were the products of *Myrothecium verrucaria* and *M. roridum*. In this communication the authors wish to deal with the production, isolation, physicochemical and biological properties and chemical structure of A-2.

When strain No. 310-1 was cultivated in a medium containing glucose 1%, peptone 0.1% and yeast extract 0.1% at 30°C for 96 hr by jar-fermentor, seven antibiotic compounds were accumulated in the culture fluid. The fluid was centrifugated at 10,000 rpm to remove the mycelium. After the supernatant, 50 liters was concentrated *in vacuo* to 5 liters, 10 liters of acetone was added to it in order to precipitate the viscous material and the supernatant was again concentrated *in vacuo* to 5 liters. The concentrate was extracted four times with ether at pH 2.0. The combined extracts were then evaporated *in vacuo* to yield a yellowish oily material, which was dissolved in a small amount of benzene and adsorbed onto a silica gel column. It was developed with 1 liter of benzene, and then with 1 liter

of benzene-ethyl acetate (95:5). The active fraction obtained by the latter development was concentrated *in vacuo* to give yellowish oily Verrucarins B, which was crystallized with ether. A-2 was found in mother liquor of Verrucarins B and recovered from the solution by repeated column chromatography. Final mother solution was treated by subsequent thin layer chromatography of Kiesel gel G with benzene-methanol (6:1). The purified fraction of A-2 was eluted with ether. The ether solution was concentrated, and crystals thus yielded were recrystallized from benzene-methanol to white prisms (1.2 mg).

A-2 had mp 147~148°C and $[\alpha]_D^{25} -14$ ($c = 1.01$, methanol). *Anal.* Found: C, 65.09; H, 7.45; O, 27.46. *Calcd.* for $C_{15}H_{26}O_6$: C, 65.12; H, 7.48; O, 27.40%; MW 350.40. The mass spectral data of A-2 showed its MW to be 350 which precisely coincided with the theoretical value. The infrared spectrum was demonstrated in Fig. 1. The ultraviolet absorption spectrum had only end absorption. A-2 was easily soluble in most organic solvents such as methanol, acetone, chloroform, ether and benzene, and sparingly soluble in hexane and petroleum ether.

A-2 exhibited specific antifungal activity against *Trychophyton asteroides* (M.I.C. 0.1 μ g/ml) and *T. interdigitale* (M.I.C. 10 μ g/ml). It was less active against yeast and other kinds of fungi such as *Aspergillus*, *Penicillium*, *Fusarium*, *Piricularia* and *Glomerella* and inactive against

FIG. 1. Infrared Spectra of A-2 (1) and 4,15-Diacetylverrucarol (2) in KBr.

1) E. Harri, W. Loeffler, H. P. Sigg, H. Stähelin, Ch. Stoll, Ch. Tamm, *Helv. Chim. Acta*, **45**, 839 (1962); B. Böhner, E. Fetz, E. Harri, H. P. Sigg, Ch. Stoll and Ch. Tamm *ibid.*, **48**, 1079 (1965).

bacteria including *Mycobacterium*.

According to the above-mentioned data and NMR spectrum, A-2 was assumed to be an acetylated Verrucarol skeleton shown in Fig. 2. In order to confirm this the comparison of A-2 and the authentic specimen was carried out. The specimen was synthesized by acetylation of Verrucarol which was made by alkali-hydrolysis of Verrucarin B. The mixture melting point test of A-2 and the specimen showed no depression. Moreover, infrared spectra of both were in perfect agreement with each other (Fig. 1).

Obviously, therefore, compound A-2 was defined to have the structure of 4,15-diacetyl-

tylverrucarol (Fig. 2), being a hitherto unknown antibiotic occurring in nature. The mold No. 310-1 was also identified taxonomically to belong to genus *Myrothecium*, but the details will be published elsewhere.

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FIG. 2. The Structure of 4,15-Diacetylverrucarol.