BOTRIC ACID, A NEW ANTIBIOTIC

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In the course of our screening program for antifungal antibiotic, a new antibiotic, named botric acid, was isolated from the culture broth of a fungous strain M-0596 belonging *Botrytis*[1].

The producing organism was submerge cultured in a medium containing 2.0 % glucose, 1.0 % peptone, 1.0 % corn steep liquor, 0.2 % KH_2PO_4, and 0.1 % $MgSO_4$ (pH 6.5) for 66 hours at 26°C.

Botric acid principally existed in the mycelia, the activity was bio-assayed using *Candida albicans* as a test organism on SABOURAUD's glucose medium.

The cultured broth (180 liters) was filtered, and the mycelial cake was extracted twice with each 20 liters of acetone. The extract was concentrated *in vacuo* to 6 liters, and the residual solution was extracted with equal volume of butylacetate. The solvent layer was treated with charcoal, and concentrated. The oily residue was dissolved in 2 liters of methanol and allowed to stand overnight at 5°C.

Crude botric acid was precipitated (10.5 g), and crystallized from hot methanol. The potasium salt was prepared as follows: the antibiotic was suspended in water and adjusted to pH 8.0 with 1 N KOH, and then lyophilized.

Botric acid is yellow crystal (plates) having a weakly acidic nature (pK'a 7.8 in 75 % dimethylformamide), and decomposes at 214~216°C. $[\alpha]_D^{24}$ −294° (*c* 0.1, $CHCl_3$).

Anal. Calcd. for $C_{25}H_{32}O_4$ (M.w. 396.53):
C 75.80, H 8.08, O 16.15
Found: C 75.33, H 8.08

The molecular weight has been confirmed by the M^+ ion at *m/e* 396 in the mass spectrum. The ultraviolet spectrum show a broad maximum at 328~338 nm ($E_{1cm}^{1\%}$ 1,410) in methanol and maxima at 235 nm ($E_{1cm}^{1\%}$ 497) and 330 nm ($E_{1cm}^{1\%}$ 497) in 0.1 N NaOH. The infrared spectrum is given in Fig. 1. The NMR spectrum (60 MHz in $CDCl_3$) is illustrated in Fig. 2.

Botric acid is soluble in acetone, ethylacetate, chloroform, dimethylformamide, dimethylsulfoxide, and alkaline water, slightly soluble in lower alcohols, and insoluble in *n*-hexane petroleum ether and water. The antibiotic gives positive reaction to LIEBERMAN-BURCHARD, SALKOWSKI, TOLLENS, MOLISCH and hydroxamic acid-$FeCl_3$, negative reaction to EHRLICH, $FeCl_3$, and 2,4-dinitrophenylhydrazine. It shows Rf values 0.76 with wet ethylacetate, 0.80 with MeOH - H_2O (4:1) and 0.35 with $CHCl_3$ - MeOH (10:1) on thin-layer chromatography (Merck, Keselgel G) detected by spraying of 0.1 % aqueous potassium permanganate solution.

Botric acid is principally active against

Fig. 1. Infrared spectrum of botric acid (KBr pellet)

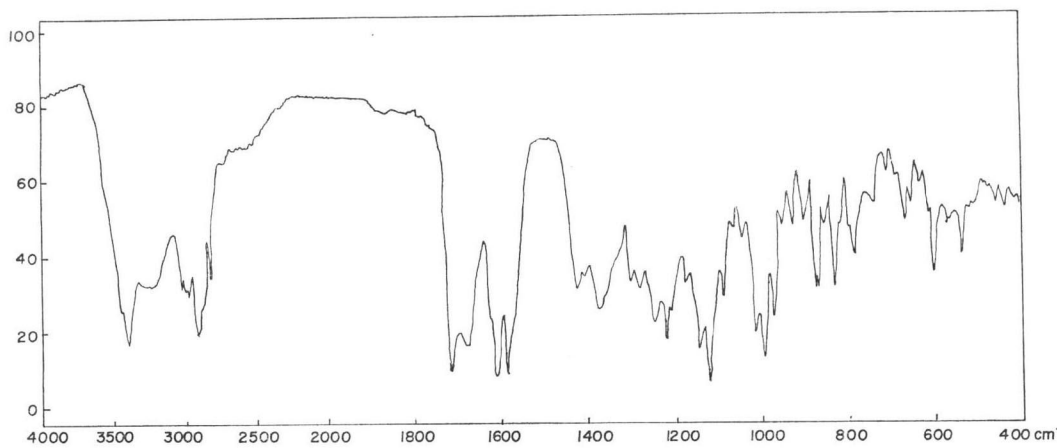
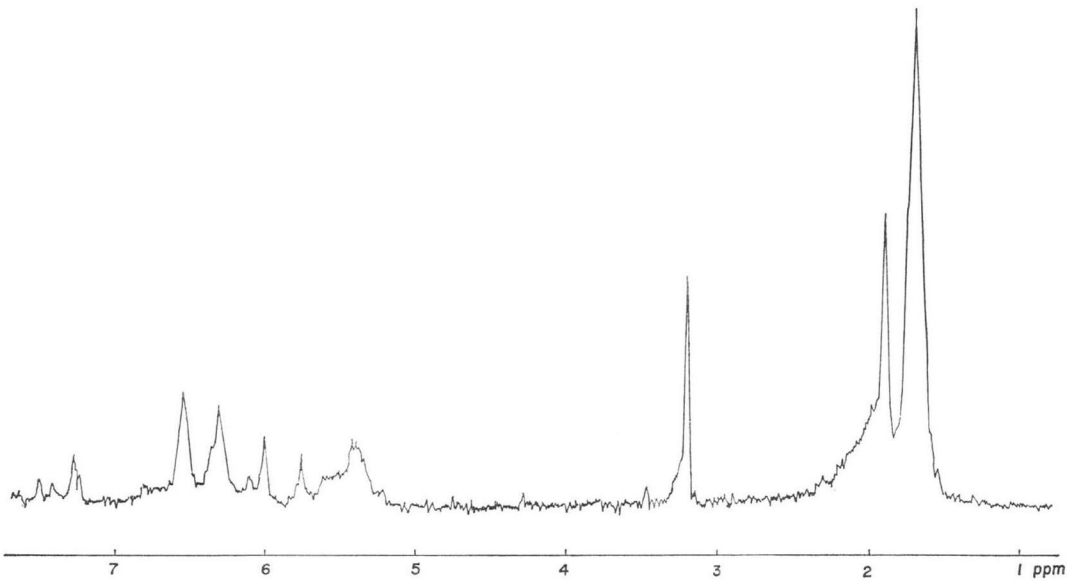


Table 1. Antimicrobial spectrum of botric acid

Test organism	Medium*	Minimum inhibitory concentration (MIC) mcg/ml	
		Botric acid	Botric acid K-salt
Bacillus subtilis PCI 219	N	50	125
Staphylococcus aureus FAD 209P	〃	100	125
Sarcina lutea	〃	>100	500
Escherichia coli NIHJ	〃	>100	>1,000
Klebsiella pneumoniae PCI 602	〃	>100	>1,000
Mycobacterium 607	〃	>100	250
Candida albicans	S	>100	6.25
〃 *tropicalis*	〃	>100	0.5
〃 *pseudotropicalis*	〃	>100	31.0
〃 *pulcherria*	〃	>100	31.0
〃 *krusei*	〃	>100	>1,000
〃 *parakrusei*	〃	>100	>1,000
〃 *guilliermondii*	〃	>100	>1,000
Cryptococcus neoformans	〃	>100	>1,000

* N: Nutrient agar, 37°C 18 hours.
S: SABOURAUD's glucose agar, 30°C 18 hours.

Fig. 2. NMR spectrum of botric acid (60 MHz in $CDCl_3$, internal reference: TMS)



Candida and practically inactive against bacteria. The antimicrobial spectrum is shown in Table 1.

Mice tolerated intraperitoneal injection with 250 mg/kg of botric acid, while intravenous injection with 125 mg/kg of the potassium salt caused delayed toxicity like streptothricin.

Many *Botrytis* species such as *B. cinerea*, *B. fabae* and *B. elliptica* have phytotoxicity[2], but no toxin has been isolated from these fungi. Botric acid showed no effect on the germination and growth of raddish and Chinese cabbage at 10~500 mcg/ml.

The physico-chemical and biological pro-

perties described above led us to conclude that botric acid is a novel antibiotic.

References

1) ELLIS, A. B.: Dematiaceous hyphomycetes. pp. 178-184, Commonwealth Mycological Institute, Kew, England, 1971
2) KATO, K.: List of important diseases and pests of economic plants in Japan. pp. 33, 150, Nihon Tokushu Noyaku Seizo Co., Tokyo 1966