NOTES

A NEW ANTIBIOTIC Y-T0678H PRODUCED BY A *CHROMOBACTERIUM* SPECIES

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In the course of our screening for new antibiotics, a Chromobacterium strain Y-T0678H isolated from a soil sample collected at Lake Kamakita in Saitama Prefecture, Japan, was found to produce a new antibiotic.

The strain Y-T0678H was an aerobic Gram-negative rod, motile with a single polar flagellum and one or two lateral flagella. The strain could grow at pH 6~8 between 10~33°C, but not at temperatures higher than 37°C and lower than 5°C. Glucose, trehalose and fructose were fermented. Casein was hydrolyzed strongly and HCN was produced. The strain also produced a violet pigment on several media. On the basis of the characteristics described above, the strain was identified as *Chromobacterium violaceum*[1,2].

The strain was cultured in 500-ml Erlenmeyer flasks containing 50 ml of a medium composed of 2.0% dextrin, 3.0% soybean meal, 1.2% $MgSO_4 \cdot 7H_2O$ and 1.0% $CaCO_3$ on a rotary shaker at 27°C for 24 hours. The antibiotic activity was monitored by paper disc assay using *Escherichia coli* K-12 as a test organism.

The fermentation broth (1,400 ml) was filtered and extracted with ethyl acetate at pH 4. After extraction, the organic layer was transferred to sodium bicarbonate solution at pH 8 and re-extracted with ethyl acetate at pH 4. The organic layer was concentrated to dryness. The crude substance (200 mg) thus obtained was purified on a preparative thin-layer chromatography (Pre-coated TLC silica gel F-254, Merck; $CHCl_3$-MeOH, 4: 1). The antibiotic was isolated as a white powder (40 mg).

Fig. 1. Structure of Y-T0678H.

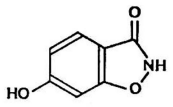

Table 1. Antimicrobial spectrum of Y-T0678H.

Test organism	MIC (μg/ml)
Bacillus subtilis ATCC 6633	>100
Micrococcus luteus ATCC 9341	>100
Staphylococcus aureus ATCC 6538P	>100
Corynebacterium xerosis	>100
Mycobacterium smegmatis ATCC 607	>100
Escherichia coli O-1	0.39
E. coli NIHJ	0.78
Klebsiella pneumoniae ATCC 10031	12.5
K. pneumoniae Y-11	3.13
Salmonella enteritidis 1891	0.78
Shigella sonnei II 37148	0.78
Proteus mirabilis IFM OM-9	3.13
P. morganii IID 602	1.56
Serratia marcescens IID 620	3.13
S. marcescens NY-10	3.13
Enterobacter cloacae 963	1.56
E. aerogenes ATCC 13048	12.5
Pseudomonas aeruginosa NCTC 10490	>100
P. putida IAM 1002	>100

MIC was determined by the serial agar dilution method with Mueller-Hinton medium. Inoculation with 10^6 cells/ml.

The physicochemical properties of Y-T0678H are as follows; acidic white powder; mp 247°C (decomp.); $[\alpha]_D^{22}$ 0° (c 0.5, MeOH); color reaction: positive ferric chloride, negative ninhydrin; HR-MS M^+ 151.02721 $C_7H_5NO_3$; *Anal.* Calcd. for $C_7H_5NO_3$: C 55.64, H 3.33, N 9.27, O 31.76, Found: C 55.38, H 3.21, N 9.06; UV_{max}^{MeOH}: 220 ($E_{1cm}^{1\%}$ 894), 250 (450), 258 sh (408), 273 sh (290), 278 sh (342), 283 (376), and 289 nm (366); IR (KBr): 3150, 1650, 1610, 1480, 1460, 1400, 1280 and 1100 cm^{-1}; ^1H NMR (DMSO-d_6): δ 6.8 (1H, d, J=1.95), 6.8 (1H, dd, J=9.03, J=1.95), 7.5 (1H, d, J=9.03), 10.3 (1H, broad) and 12.0 (1H, broad); ^{13}C NMR (DMSO-d_6): δ 165.5 (s), 164.9 (s), 160.5 (s), 122.0 (d), 113.0 (d), 106.9 (s) and 95.1 (d). The analytical and spectroscopic data

of Y-T0678H indicated above, suggested that the structure was 6-hydroxy-3-oxo-1,2-benzisoxazolin (I) as shown in Fig. 1. The structure was confirmed by actual synthesis starting from methyl-2,4-dihydroxybenzoate by a similar method suggested by BÖSHAGEN[3]. These two samples of I gave identical spectral data and similar antimicrobial activity. The antimicrobial activity of Y-T0678H was shown in Table 1. Y-T0678H exhibits a selective activity against Gram-negative bacteria. The acute toxicity (LD_{50}) in mice of Y-T0678H is about 1,560 mg/kg (i.v.).

Acknowledgment

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References

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