Short Communication

Xanthostatin, a New Antibiotic

Xing-Chun Cheng,† Tsuyoshi Kihara, Hiroo Kusakabe, Ren-Pin Fang,‡ Zher-Fu Ni,† Yin-Chu Shen,‡ Keido Ko, Isamu Yamaguchi and Kiyoshi Isono*

Riken, The Institute of Physical and Chemical Research, Wako-shi, Saitama 351-01, Japan
†Shanghai Pesticide Research Institute, Shanghai, China

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A strain of Streptomyces isolated from a soil sample collected in Jiangsu Province, China, was found to produce a new antibiotic selectively active against Xanthomonas sp. The strain has not yet been identified but the antibiotic was designated as xanthostatin because of its selective activity against Xanthomonas sp. Fermentation was carried out at 28°C for 96 hr in a jar fermentor containing 18 liters of a medium, which was composed of glucose (2%), soluble starch (1%), meat extract (0.1%), dry yeast (0.4%), soybean flour (2.5%), NaCl (0.2%), and K₂HPO₄ (0.005%).

The filtered broth (25 liters) was extracted with ethyl acetate at pH 4, and the extracts were concentrated to dryness, giving 4.8 g of an oily residue that was crystallized from methanol. The crystals were collected by filtration, washed with methanol (400 mg), and further purified by preparative HPLC (Nucleosil 5C₁₈, 20 mm × 250 mm; solvent, methanol–H₂O = 8:2; speed, 6.0 ml/min). An active peak (12 min retention time) was collected, concentrated to dryness and the residue was recrystallized from methanol, affording 150 mg of pure crystals of the antibiotic.

Xanthostatin was formed as colorless crystals with a melting point of 157~160°C with decomposition. It was optically active, [α]₀¹⁵ = −76° (c 0.5, MeOH), and soluble in methanol, ethyl acetate and chloroform, but hardly soluble in benzene, hexane and water. It was a neutral compound having no titrable group, and was stable in neutral and acidic conditions, but unstable in an alkaline solution. From elementary analysis and FD mass spectroscopy [M + H]⁺ 895, (M + Na)⁺ 918, the following molecular formula has been established. Calcd. for C₄₄H₆₁N₇O₁₃. H₂O: C, 57.83; H, 6.90; N, 10.73; O, 24.53. Found: C, 58.14; H, 6.77; N, 10.70; O, 24.39. The antibiotic had a UV spectrum of λ max (ε cm⁻¹) : 230 (90), 265 sh (7.3), 268 sh (8.1), 274 (8.5) and 282 (7.1), which is characteristic of tyrosine (Fig. 1), the spectrum remaining virtually unchanged in acidic and alkaline methanol. The IR spectrum is shown in Fig. 2, main absorption bands appearing at wavelengths of 3300, 1725, 1525, 1500, 1450, 1295, 1245, 1195, 1080, 1050, 1020, 825, 745 and 695 cm⁻¹. The presence of ester and amide bonds was indicated. The ¹H NMR spectrum is shown in Fig. 3, giving positive Rydon-Smith and Dragendorf tests, but a negative ninhydrin test. An amino acid analysis of

* To whom correspondence should be addressed.
the hydrolysate (6 N HCl, 120°C, 16 hr) gave threonine, serine, glycine and tyrosine in an approximate molar ratio of 2:1:1:0.6, and two additional unidentified amino acids.

Xanthostatin was specifically inhibitory to Xanthomonas oryzae and Xanthomonas citri (Table I). Other bacteria and fungi tested were not affected, and mice tolerated an intraperitoneal injection of 400 mg/kg of the antibiotic.

Although ascamycin1) and xanthocidin2) are known to selectively inhibit Xanthomonas sp., no peptide antibiotic has before been known that is selectively active to Xanthomonas sp. In
Addition, xanthostatin was clearly different from the peptide antibiotics containing tyrosine, e.g., enduracidin,3) gratisin,4) mycobacillin,5) arphamenine B6) bacillomycin F,7) chaetomacin,8) iturins,9) mycocerin,10) A43F,11) and K-26.12)

### Table 1. Antimicrobial Activity of Xanthostatin

The conventional agar dilution method was used. Potato-sucrose agar medium was used for yeasts and fungi, and bouillon agar for bacteria.

<table>
<thead>
<tr>
<th>Test organism</th>
<th>Minimal inhibitory concentration (mcg/ml)</th>
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<tbody>
<tr>
<td>Xanthomonas oryzae IFO 3312</td>
<td>2</td>
</tr>
<tr>
<td>Xanthomonas citri IFO 3781</td>
<td>4</td>
</tr>
<tr>
<td>Escherichia coli IFO 3301</td>
<td>&gt;1,000</td>
</tr>
<tr>
<td>Salmonella typhimurium TV 119</td>
<td>&gt;1,000</td>
</tr>
<tr>
<td>Staphylococcus aureus 209P IFO 12732</td>
<td>&gt;1,000</td>
</tr>
<tr>
<td>Bacillus subtilis IFO 3513</td>
<td>&gt;1,000</td>
</tr>
</tbody>
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### REFERENCES