Isolation and cytotoxicity of puberulonic acid from *Penicillium resticulosum*

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井川美好*¹・辰野高司*²・角田 廣*²・梅田 誠*³: *Penicillium resticulosum*
からのプベルロン酸の分離とその細胞毒性について

Puberulonic acid (I) was isolated by Birkinshaw and Raistrick¹⁾ from *Penicillium puberulum* Bainier and *Penicillium aurantio-virens* Biourge grown on Czapek-Dox glucose medium and its

structure determined by Aulin-Erdtman²⁾. In this paper we wish to report the isolation of puberulonic acid from *Penicillium resticulosum* Birkinshaw, Raistrick & Smith and the cytotoxic properties of the puberulonic acid against HeLa cells. Previous studies have indicated the cytotoxic effects of synthetic puberulonic acid against HeLa cells³⁾.

Penicillium resticulosum was grown in Petri dishes on Czapek-Dox medium containing 5% sucrose, 0.3% yeast extract, and 2% agar. At the end of 6 days at room temperature, when the agar side of the mold colony was a brilliant yellow, the contents from 40 plates were extracted with 900 ml of acetone in a blender. The mixture was filtered by suction, giving an orange-yellow filtrate, and the residue extracted again with 300 ml of acetone. The extracts were combined and concentrated to dryness *in vacuo*. The orange residue was treated with a small volume of hot methanol, filtered, and the insoluble residue washed with a small volume of methanol. An orange methanol-insoluble powder (369 gm) was obtained. The powder (77 mg) was dissolved in 5 ml of 5% NaHCO₃ and filtered. The filtrate was acidified to pH 1–2 with concentrated HCl and extracted repeatedly with 15 ml portions of ethyl acetate until the ethyl acetate

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was almost colorless, 6 extractions being necessary. The extracts were combined, filtered, and concentrated *in vacuo* to dryness, yielding 51 mg of a crystalline residue, which was recrystallized from ethyl acetate to give 16 mg of brilliant yellow crystals.

Analysis: Found- C, 48.6%; H, 2.2%. Calculated for $C_9H_4O_7$ (puberulonic acid)- C, 48.2%; H, 1.8%.

The crystals melted at 290–300°. Birkinshaw and Raistrick¹⁾ report 296°d for puberulonic acid. The IR spectrum (KBr pellet) showed very strong anhydride C=O stretching bands at 1820 and 1760 cm^{-1} , and the spectrum agreed with the IR spectrum reported by Aulin-Erdtman and Theorell⁴⁾ for puberulonic acid. The UV-visible spectrum in 1 M HCl was identical with that reported by Aulin-Erdtman²⁾. It can therefore be concluded that the bright yellow pigment produced by *P. resticulosum* is puberulonic acid.

Cytotoxicity tests against HeLa cells were carried out as previously described³⁾. At 100 $\mu\text{g}/\text{ml}$, cell damage was scored as 4 (complete cytolysis); at 32 $\mu\text{g}/\text{ml}$, as 3.5; at 10 $\mu\text{g}/\text{ml}$, as 2; and at 3.2 $\mu\text{g}/\text{ml}$, as 0 (no cell damage). Morphological changes observed were enlarged nuclei and nucleoli. A few multinuclear cells were observed. Although puberulonic acid appeared to be more toxic than rubratoxin B, which also contains the 5-membered anhydride ring, the morphological changes were less conspicuous than those caused by B.

Summary

Puberulonic acid was isolated from a culture of *Penicillium resticulosum* and shown to possess cytotoxic activity against HeLa cells.

References

- 1) Birkinshaw, J. H., and Raistrick, H.: *Biochem. J.* **26**, 441 (1932).
- 2) Aulin-Erdtman, G.: *Acta Chem. Scand.* **5**, 301 (1951).
- 3) Umeda, M., Yamashita, T., Saito, M., Sekita, S., Takahashi, C., Yoshihira, K., Natori, S., Kurata, H., and Udagawa, S.: *Japan. J. Exp. Med.* **44**, 83 (1974).
- 4) Aulin-Erdtman, G., and Theorell, H.: *Acta Chem. Scand.* **4**, 1490 (1950).