NOTES

PRUMYCIN PRODUCED BY *BACILLUS CEREUS*

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The isolation of prumycin from cultured broths of *Streptomyces kagawaensis* has been reported by HATA *et al.*[1] It inhibits the growth of some bacteria and fungi,[1] and shows antitumor action.[2] The structure determined by ŌMURA *et al.*[3,4] to be 4-D-alanylamino-2-amino-2,4-dideoxy-L-arabinose.

We isolated this antibiotic from cultured broths of a strain BMG366-UF5 which was isolated from a soil sample collected at Suginami, Tokyo, and classified as *Bacillus cereus*.[5] *Bacillus cereus* BMG366-UF5 was deposited with the Fermentation Research Institute, Agency of Industrial Science and Technology, Japan, as FERM P-6395.

The spores on an agar slant were suspended with sterilized water (120 ml). The suspension (1.2 ml) was inoculated into a medium (110 ml) containing 3.0 % glycerol, 2.0 % fish meal and 0.2 % $CaCO_3$ (adjusted to pH 7.4 with 4 M NaOH) in a 500-ml baffled Erlenmeyer flask, and the culture was grown at 27°C for 3 days on a rotatory shaker (180 r.p.m.).

The cultured broth (20 flasks) was filtered and the antibiotic in the filtrate (2,000 ml, pH 6.8, 404 μg/ml determined by the cylinder-plate method against *Escherichia coli* K-12) was adsorbed on a column of Amberlite IRC-50 (40 % Na^+, 230 ml). After washing the column with water (1,000 ml), the antibiotic was eluted with 0.4 M HCl and the active eluate (390 ml) was concentrated to dryness (13 g, 40 μg/mg). The residue was extracted with methanol (20 ml) to remove the salt, and the extract was concentrated to dryness (7.25 g, 81 μg/mg). The residue was dissolved in 0.2 M NaCl (6 ml) and the antibiotic in the solution was adsorbed on a column of CM-Sephadex C-25 (200 ml, equilibrated with 0.2 M NaCl). After washing the column with 0.2 M NaCl (600 ml), the antibiotic was eluted with 0.4 M NaCl and the active eluate (390 ml) was concentrated to dryness. The residue was extracted with methanol (20 ml) and the extract was concentrated to give a crude powder (1.24 g, 343 μg/mg). The powder was further purified by readsorption on a column of CM-Sephadex C-25 (90 ml, equilibrated with 0.4 M NaCl) followed by elution with 0.4 M NaCl. The active eluate (200 ml) was concentrated, the residue was extracted with methanol (15 ml) and the extract was concentrated to yield a powder (871 mg, 505 μg/mg). The methanol solution (10 ml) of the powder was passed through a column of Sephadex LH-20 (90 ml, developed with methanol) and the active eluate (25 ml) was concentrated to give a powder (629 mg, 706 μg/mg, 55 % yield from the broth filtrate) of prumycin hydrochloride. Crystallization from a methanol solution (1 ml) of the powder (310 mg) gave the β-anomer of prumycin dihydrochloride (40 mg, 1,000 μg/mg), mp 189~192°C (decomp.), $[\alpha]_D^{25}$ +111° (*c* 1, methanol) [lit.[4] mp 198~200°C (decomp.), $[\alpha]_D^{20}$ +115° (*c* 0.5, methanol)]. All spectral data of the antibiotic were identical with those of prumycin.

References

1) HATA, T.; S. ŌMURA, M. KATAGIRI, K. ATSUMI, J. AWAYA, S. HIGASHIKAWA, K. YASUI, H. TERADA & S. KUYAMA: A new antifungal antibiotic, prumycin. J. Antibiotics 24: 900~901, 1971
2) OKUBO, S.; N. NAKAMURA, K. ITO, H. MARUMO, M. TANAKA & S. ŌMURA: Antitumor activity of prumycin. J. Antibiotics 32: 347~354, 1979
3) ŌMURA, S. & M. TISHLER: Structure of prumycin, a 2,5-diamino-2,5-dideoxypentose-containing antibiotic. J. Chem. Soc., Chem. Commun. 1972: 633~634, 1972
4) ŌMURA, S.; M. KATAGIRI, K. ATSUMI, T. HATA, A. A. JAKUBOWSKI, E. B. SPRINGS & M. TISHLER: Structure of prumycin. J. Chem. Soc., Perkin Trans. I 1974: 1627~1631, 1974
5) BUCHANAN, R. E. & N. E. GIBBONS (*ed*): BERGEY's Manual of Determinative Bacteriology, 8th Ed., p. 534, The Williams & Wilkins Company, Baltimore, 1974