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Short Communication

Nebularine from a novel *Microbispora* sp.

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SUMMARY

Nebularine has now been isolated from a novel *Microbispora* sp., identified as a new mesophilic species. An efficient method for the isolation of nebularine using Droplet Counter Current Chromatography is described.

Isolation of nebularine from fungal [7] and streptomycete [5] fermentations has been reported previously. The antibiotic structure has been confirmed synthetically [2,3] and shown to be 9-β-D-ribofuranosyl purine. Nebularine has now been isolated from a novel *Microbispora* sp. described herein. An efficient method for the preparative separation of this compound has been accomplished using Droplet Counter Current Chromatography (DCCC) [4].

MATERIALS AND METHODS

Strain, media and inoculum conditions

The producing culture, SCC 1779, was isolated from a soil sample collected in Thailand. The strain was characterized by good growth on most organic media, the formation of tan to brown vegetative mycelial pigments, faint yellow-brown diffusible pigments and abundant white to pink aerial mycelia. The aerial hyphae formed characteristic, closely arranged longitudinal pairs of sessile, non-motile spores. Whole cell analysis by the methods of Becker et al. [1] and Lechevalier [6] indicated the presence of meso-diaminopimelic acid and madurose as the major constituents. Maximum growth occurred between 27°C and 35°C; poor growth was observed at 40°C. Glucose, rhamnose and trehalose were utilized. Tyrosine, starch and casein were hydrolyzed. Good growth occurred in the presence of 50 μg/ml of nalidixic acid, novobiocin and rifamycin. The strain was identified as a new mesophilic species of *Microbispora* designated *Microbispora* sp. SCC 1779.

Microbispora sp. SCC 1779 was inoculated (5%, v/v) into 50 ml seed medium consisting of (w/v): beef extract 0.3%, tryptone 0.5%, yeast extract 0.5%, cerelose 0.1%, starch 2.4% and $CaCO_3$ 0.2% in a 250 ml Erlenmeyer flask. The resulting seed cultured broth was incubated for 48 h at 30°C on a rotary shaker operating at 300 rpm then used to inoculate (5%) into a second seed stage using the same medium. The second seed stage (25 ml) was

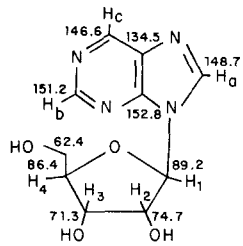
inoculated into 400 ml of the production medium composed of yeast extract 0.5%, fish-peptone 0.6%, dextrin 4.0%, cerelose 2.2%, $MgSO_4 \cdot 7H_2O$ 0.2%, K_2HPO_4 0.08%, $CoCl_2$ 10^{-6} M in a 2 l Erlenmeyer flask. Before sterilization the pH was adjusted to 7.2 and $CaCO_3$ 0.4% was added. The fermentation was run for 96 h at 30°C on a 300 rpm shaker. Production and isolation of the antibiotic were followed by disc diffusion assay against *Candida albicans*.

Isolation

The filtered broth (7 liters) was extracted with H_2O-saturated *n*-BuOH (2×). The concentrate (9 g) was treated with a H_2O:MeOH (1:9) mixture. The active aqueous MeOH filtrate was concentrated to a residue (5 g). A portion of this material (500 mg) was chromatographed by DCCC. DCCC was performed on a Model DCC-A-300 (Tokyo Rikakikai Co., Tokyo, Japan) with 300 glass columns, 40 cm × 2 mm (i.d.). In a typical run the solvent system $CHCl_3$:MeOH:H_2O (7:13:8) was used in the ascending mode (i.e., aqueous layer, mobile phase) at a flow rate of 6 ml/h, collecting 3 ml fractions. The antifungal fractions, 51–64, were pooled, concentrated and lyophilized to yield 148 mg of nebularine.

RESULTS AND DISCUSSION

The melting point, optical rotation and mass spectroscopic data for the weakly basic solid are consistent with published data [5]. The physical properties are as follows: $[\alpha]_D^{26}$ (*c* 1.0, H_2O) −43.9°; reported [3] $[\alpha]_D^{25}$ (*c* 2, H_2O) −46.8°; UV (H_2O λ_{max} 262 nm, ε 6250), with no shift upon addition of acid or base; m.p. 180–182°C, reported 181–182°C [2], 180–181°C [5]. Acid hydrolysis (2N HCl, 100°C) gave ribose and purine identified on t.l.c. by comparison with authentic samples. FAB-mass spectrometric analysis of nebularine gave a protonated molecular ion, M + H^+ 253 and a sodiated ion, *m/z* 275 corresponding to a molecular weight of 252. ^1H and ^{13}C-NMR spectra were obtained in D_2O on a Varian XL-200 instrument. The data are shown in Fig. 1 and are in agreement with the assigned structure.



PROTON	δppm	J(Hz)
H-1	6.18 d	2
H-2	4.82 br	2,5
H-3	4.43 dd	5,6
H-4	4.24 m	
H-5(2)	3.80 brd	
H_a	8.63 s	
Hb	8.65 br	
Hc	8.80 brs	

Fig. 1. Structure of nebularine, together with ^1H and ^{13}C-NMR assignments.

In conclusion, we believe this is the first report of nebularine being produced by a *Microbispora* sp. The separation methods used to isolate nebularine constitute a novel approach and are more efficient. We were able to obtain essentially pure compound (90% recovery) by a one-step purification, in contrast to the previously reported isolation [5]. The complete description on the taxonomy of the novel *Microbispora* sp. will be reported elsewhere (A.C. Horan, manuscript in preparation).

REFERENCES

1 Becker, B., M.P. Lechavalier and H.A. Lechevalier. 1965. Chemical composition of cell-wall preparations from strains of various form-genera of aerobic actinomycetes. Appl. Microbiol. 13: 236–243.

2 Brown, G.B. and V.S. Welisky. 1953. Synthesis of 9-β-D-ribofuranosyl purine and the identity of nebularine. J. Biol. Chem. 204(2): 1019–1024.

3 Hashizume, T. and H. Iwamura. 1966. A new synthesis of nebularine and 7-D-ribofuranosyl purine. Tetrahedron Lett. 643–648.

4 Hostettmann, K. 1980. Droplet counter-current chromatography and its application to the preparative scale separation of natural products. Planta Medica 39: 1–18.

5 Isono, K. and S. Suzuki. 1960. 9-β-D-Ribofuranosyl purine from a Streptomycete. J. Antibiot. 13A: 270–272.

6 Lechavalier, M.P. 1968. Identification of aerobic actinomycetes of clinical importance. J. Lab. Clin. Med. 71: 934–944.

7 Lofgren, N. and B. Luning. 1953. Structure of nebularine. Acta Chem. Scand. 7: 225.