BRIEF REPORT

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The Antibiotic PSX-1 Produced by *Pennicilium stipitatum* Is Identical with Botryodiplodin

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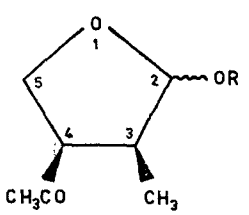
ABSTRACT. Antibiotic PSX-1 exhibiting antitumour activity, formerly isolated from the filtrate of a culture of *Penicillium stipitatum*, was shown to be identical with botryodiplodin.

Fuska and coworkers (1974) isolated the antitumour antibiotic PSX-1 (**1**) from a filtrate of *Penicillium stipitatum* THOM. In addition to the metabolite PSX-1, the culture also produced a compound designated PSX-2 and shown to be identical with duclauxin (Kuhr *et al.* 1973), stipitatic acid, stipitatonic acid (Fuska *et al.* 1975), stipitalide (Holík and Kuhr 1973) and 4-hydroxy-6-methyl-2*H*-pyran-2-one ("triacetic acid lactone"; Brenneisen *et al.* 1964). Using Czapek-Dox medium containing 5 % (*W*/v) glucose and 0.3 % (*W*/*V*) peptone as the sole nitrogen source, the culture produced only compound **1**. The mycelium-free filtrate (pH 4) was extracted with a chloroform—2-propanol (3 : 1) mixture, the extract was concentrated and the syrupy residue was chromatographed on a silica gel column (*Lachema*, 100—160 μm) and eluted by chloroform—methanol (10 : 1) mixture. Compound **1** was crystallized from a diethyl ether—hexane (1 : 1) mixture. The crystals were purified by sublimation (10 °C, 101.3 kPa, 30 d).



1 R=H
2 R=CH_3CO

The melting point was estimated by using a Kofler stage; UV, IR and ^{1}H NMR spectra were recorded using a Specord UV VIS-, a Specord IR 71- and a Bruker AM 300 apparatus, respectively. The samples were dissolved in $CDCl_3$.

Compound **1** was obtained as colourless crystals; melting point 44—45 °C; R_F 0.40 (Silufol UV_{254}; chloroform—methanol, 10 : 1). The calculated and the found proportions of carbon and hydrogen for $C_7H_{12}O_3$ (144.2) were 58.30, 8.39 and 58.26, 8.43 %, respectively. UV: λ_{max}^{MeOH} (ε) 270 nm (64.8 m^2/mol). IR ($CHCl_3$): 3595, 3400, 3007, 1708, 1461, 1371, 1358, 1180, 1094, 1062, 990 and 917 cm^{-1}. MS (80 °C, 300 μA, 3.7 aJ): 144 (1.4), 128 (23), 126 (64), 98 (27), 87 (57), 85 (32), 83 (100). According to its ^{1}H NMR spectrum, compound **1** was a mixture of two anomers. By acetylation in an acetanhydride—pyridine mixture, acetate **2** was prepared: melting point 66—67 °C; R_F 0.73; $[\alpha]_D^{20}$ —99° (*c* 0.1; $CHCl_3$). The calculated and the found proportions of carbon and hydrogen for $C_9H_{14}O_4$ (186.2) were 58.05, 7.58 and 58.12, 7.51 %, respectively. IR ($CHCl_3$): 3019, 1734, 1711. MS (%): 126 (45), 98 (31), 87 (54), 85 (36), 83 (100). ^{1}H NMR ($CDCl_3$, ppm): 0.92 (3H, d, J = 7.7 Hz), $C_{(3)}$-CH_3; 2.06 (3H, s), CH_3COO-$C_{(1)}$; 2.25 (3H, s), $C_{(4)}$-$COOCH_3$; 2.72 (m, 1H), $C_{(3)}$-H; 3.82 (m, 1H, $J_{3,4}$ = 7.2 Hz) $C_{(4)}$-H; 4.25 (m, 2H), $C_{(5)}$-H; 5.95 (s, 1H), $C_{(2)}$-H. Comparison of these data with those published for botryodiplodin (Arsenault *et al.* 1969; McCurry and Abe 1973) suggests that both compounds are identical.

Botryodiplodin has been isolated from *Botryodiplodia theobromae* (Sen Gupta *et al.* 1966), *Penicillium carneo-lutescens* (Fujimoto *et al.* 1980), *P. roqueforti* (Moreau *et al.* 1982), *Lacunospora* sp., *Triangularia* sp. and *Zopfiella* sp. (Naito *et al.* 1979). The antitumour effect of botryodiplodin including its mechanism has also been discused (Douce *et al.* 1982; Moulè *et al.* 1981, 1982, 1984 *a,b*). Compound **1** inhibited RNA and DNA synthesis in various types of tumours *in vitro* (Fuska *et al.* 1974) and tumour growth *in vivo* (Fuska and Fusková 1976). Its toxicity for normal and rat fibroblasts was rather low at a concentration of 2.5 mg/L. Cytotoxic and cytopathic effects were more pronounced with tumour-type fibroblasts, especially with fibroblasts transformed with Rous sarcoma virus, which is in keeping with the priority of RNA synthesis inhibition (Fuska *et al.* 1976).

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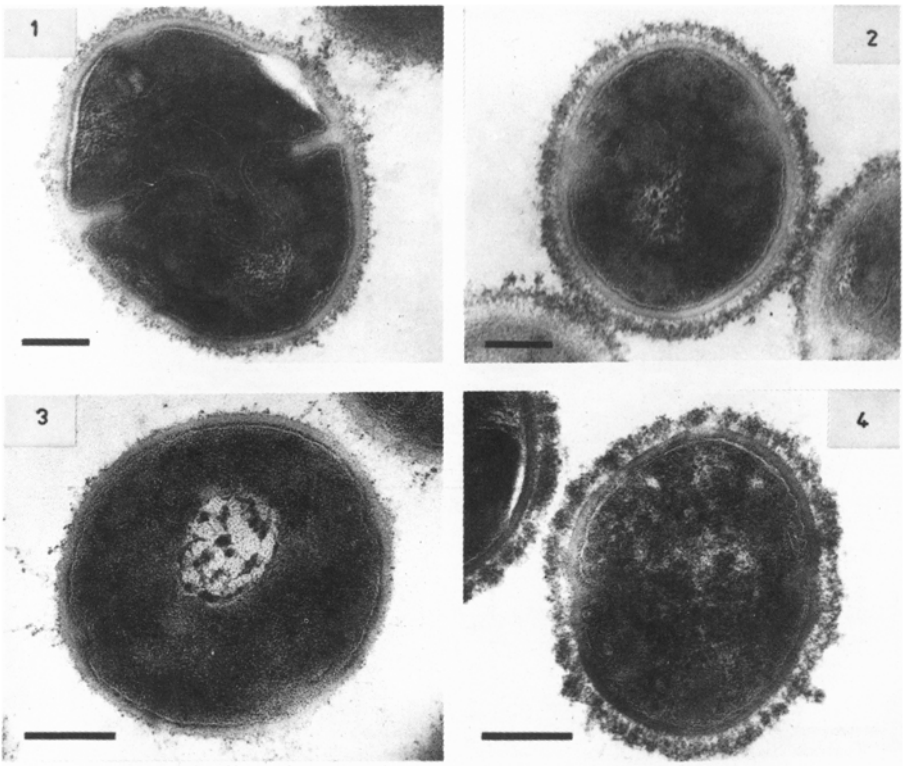

PLATE I. Group B streptococci before transfer; 1 strain A without incubation with type antiserum, 2 strain A after incubation with type antiserum, 3 strain D without incubation with type antiserum, 4 strain D after incubation with type antiserum; ultrathin sections, bars represent 0.2 μm.

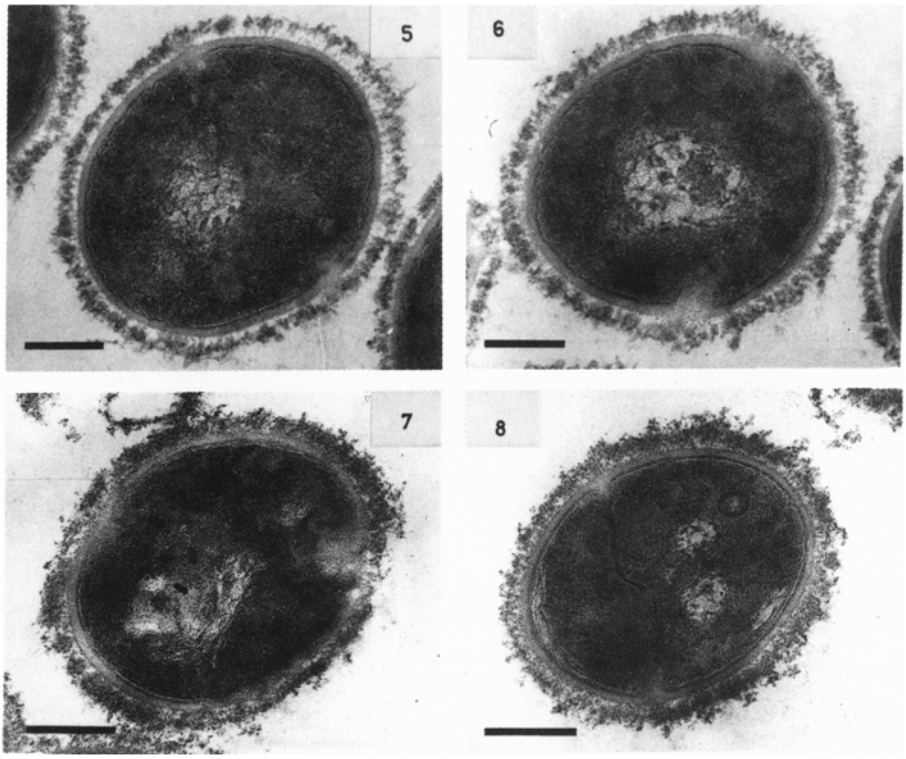

PLATE II. Group B streptococci after repeated transfers or storage and after incubation with type antisera; **5** strain A after two transfers, **6** strain A after storage, **7** strain D after two transfers, **8** strain D after storage; ultrathin section, bars represent 0.2 μm.

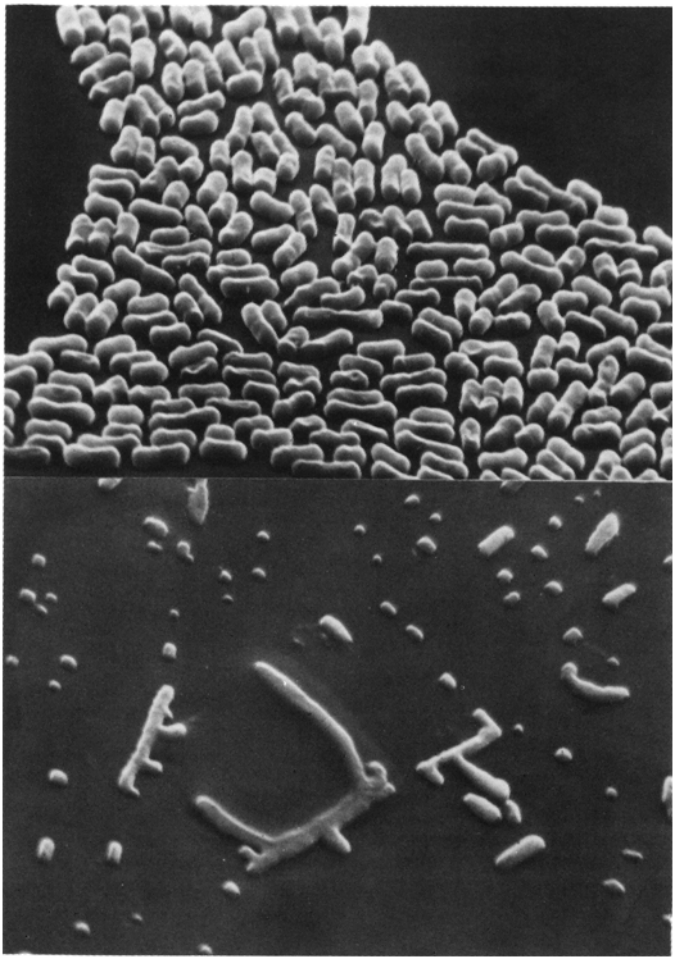

PLATE 1. Effect of 2-(iodomethyl)-5-nitrofuran (**1**) on the morphology *E coli* (SEM, growth at 37 °C for 1 d);;*top*: absence of test compound; *bottom*: presence of test compound (5 mg/L) showing unusual forms; note elongation, branching and atypical rod shapes.

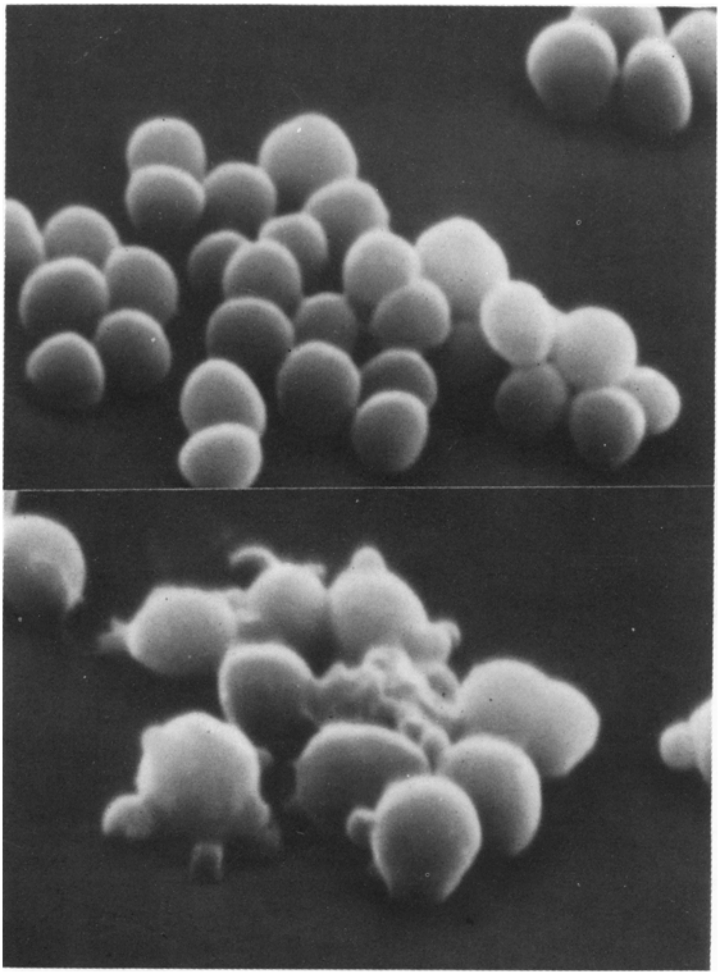

PLATE 2. Effect of 2-(iodomethyl)-5-nitrofuran (1) on the morphology of *S. aureus* (SEM, growth at 37 °C for 1 d); *top*: absence of test compound; *bottom*: presence of test compound (5 mg/L) showing multibud formation.

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