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BIOSYNTHESIS OF PATULIN IN PENICILLIUM URTICAE: IDENTIFICATION OF ISOPATULIN AS A NEW INTERMEDIATE

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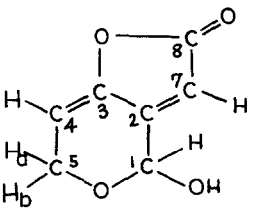
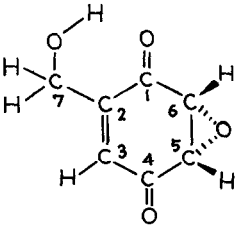
Patulin (1), a potent antibiotic produced by Penicillium and Aspergillus species[1] is known as a tetraacetate-derived secondary metabolite. It has been proposed that the biosynthetic route is via m-cresol, m-hydroxybenzylalcohol, m-hydroxybenzaldehyde, gentisaldehyde[2] and phyllostine (2).[3] We now wish to describe the isolation and structural determination of the new metabolite named isopatulin (3) and also the elucidation of the post-gentisaldehyde portion of the patulin pathway

Penicillium urticae NRRL 2159A accumulated 13 mM of 1[4] but could not accumulate 3, whereas a patulin-deficient mutant, S15 derived from 2159A accumulated a small amount of 3. The best way to accumulate 3 was to use the immobilized cells. P. urticae 2159A cells were embedded in acrylamide gels and then gels were cut to small particles (30-60 mm^3). Phyllostine (2) was added to 0.05 M phosphate buffer (pH 6.5) containing gel particles, and the suspension was incubated at 30°C for 2 hr. Its incubation mixture removed from gels was acidified to pH 2 and extracted with ethyl acetate. The crude combined extracts were applied onto preparative TLC plates (Chloroform : Ethyl acetate : Ethyl ether 5 : 2 : 1), and an isopatulin band (3, Rf 0.30) which was visible under UV, was scraped from plates and extracted with ethyl acetate. The combined extracts were evaporated to give 78 mg of crystalline 3 (prisms from ethyl acetate, m.p. 88-90°C; 1 spot on TLC) starting from 600 mg of synthetic (±)-phyllostine.[5] Assuming that this immobilized system is active only to (-)-enantiomer of (±)-phyllostine, the yield of the transformation was 26% and no patulin (1) was detected in this reaction mixture.

The physical and spectral data of isopatulin (3) are as follows:
$C_7H_6O_4$ (mol wt 154; Anal. Calcd: C, 54.55; H, 3.92. Found: C, 54.79; H, 4.15); UV: λ_{max}^{MeOH} 269 nm (ε 12800); ir: ν_{max}^{nujol} 3400 cm^{-1}, 1780, 1755, 1675, 1610, 1440, 1400, 1280, 1225, 1175, 1110, 1070, 1040, 1000, 980, 900, 845, 820, 795; pmr: δ(CD_3COCD_3) 3.08 (s, 5-OH), 4.74 (dd, J_{a-b} =



1



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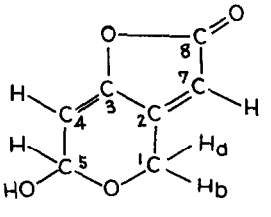

3

16.9 Hz and J_{b-7} = 1.2 Hz, 1-Hb), 5.04 (dd, J_{a-b} = 16.9 Hz and J_{a-7} = 2.2 Hz, 1-Ha; Shifts of Ha and Hb may be interchanged.), 5.72 (m, 4-H), 5.91 (m, 5-H), 6.01 (m, 7-H); cmr: $\delta(CD_3COCD_3)$ 58.2 (t, 1-C), 90.4 (d, 5-C), 109.0, 110.8 (each d, for 4- and 7-C), 150.2 (s, 2-C), 153.4 (s, 3-C), 169.6 (s, 8-C); mass: m/e 154 (M^+), 136, 126, 112, 97, 67, 39. A CD spectrum of 3 showed that it was optically inactive. The pmr and ir spectra of 3, when compared with those of 1,[3] indicated that the lactone and hemiacetal rings were similarly present in 3. Also the cmr and mass spectra of 3 supported 3 is very similar to 1. Only difference in pmr spectra of both compounds was two coupling constants assigned as J_{a-7} = 2.2 Hz and J_{b-7} = 1.2 Hz in 3, and those assigned as J_{a-4} = 2.9 Hz and J_{b-4} = 3.8 Hz in 1.[3] Those weak coupling constants in 3 suggested that 1-Ha and Hb had long range couplings with 7-H, whereas 5-Ha and Hb in 1 had vicinal couplings with 4-H. These facts suggest that isopatulin (3) is an isomer of patulin (1), having the OH group at C-5 in contrast with 1 which has the OH group at C-1.

The association of isopatulin (3) with the patulin pathway was investigated using a patulin-deficient mutant, P. urticae P3 which was blocked between phyllostine (2) and isopatulin (3), and also blocked at or before the step(s) of 6-methylsalicylic acid synthetase. Isopatulin (3, final concentration of 1 mM) was added to a washed cell suspension of mutant P3 and samples were withdrawn 1 min, 1 hr, 3 hr and 5 hr after addition. After filtration each sample was extracted, concentrated and spotted on TLC, and the quantity was measured with the dual-wavelength TLC scanner CS 900. 3 was not detected by 1 hr and conversions of 14%, 28% and 27% to 1 were obtained by 1 hr, 3 hr and 5 hr, respectively. Control experiments (no addition of 3) indicated that none of patulin-pathway metabolites was detected in the cell suspension of mutant P3. In order to identify 1 transformed from 3, 1 formed by the cell suspension of mutant P3 was isolated, purified and characterized. As well as the chromatographic pattern (Rf 0.33 in Chloroform : Ethyl acetate : Ethyl ether 5 : 2 : 1), its UV, mass and ir spectra were completely identified with those of authentic patulin. These facts strongly support that the isopatulin (3) was a true intermediate for the biosynthesis of patulin (1) in P. urticae, and a hydride shift was proposed in a mechanism of conversion of 3 into 1. The structure of isopatulin (3) and its role as an intermediate in the patulin pathway reasonably explain the probable mechanism in which the cleavage of 4-C and 5-C bond of phyllostine (2) is necessarily involved in the step of patulin skeleton formation without any redox reaction.

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