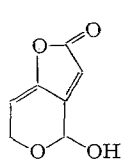
Desoxypatulinic Acid from a Patulin-Producing Strain of *Penicillium patulum*

Desoxypatulinic acid, previously obtained by synthesis[1] and by hydrogenation of patulin[2], has not been reported as a natural product. We have now isolated it from cultures of *Penicillium patulum* Bainier (*P. urticae* Bainier), a known source of many fungal metabolites, including patulin[3,4].

P. patulum[5], strain No. 562 isolated from a mixed feed suspected of causing death of a number of cattle, was grown at room temperature in 2.8-liter Fernbach flasks each containing 200 ml of yeast extract (2%)-sucrose (15%) liquid medium. After 9–11 days incubation, the medium was extracted with 3 equal volumes of ethyl acetate. The solvent was evaporated, and the residue dissolved in warm benzene and chromatographed on a column of silica gel. Elution with benzene-ethyl acetate (9:1, v/v) yielded patulin (I) (124 mg per Fernbach flask), m.p. 110–111 °C after recrystallization from benzene; it was identified by comparison with standard material (mixed m.p., UV- and IR-spectra, and thin-layer chromatography (TLC)). Further elution of the column with benzene-ethyl acetate (85:15, v/v) gave fractions containing griseofulvin (identified by comparative TLC). A more polar compound (80 mg crystalline material per Fernbach flask) was eluted by benzene-ethyl acetate (85:15 to 75:25, v/v). It was purified by crystallization from *iso*-propyl ether-*n*-hexane and toluene followed by sublimation. The crystals, m.p. 115–115.5 °C, were soluble in water (to give an acidic pH) and aqueous $NaHCO_3$ (with effervescence). The molecular formula was $C_7H_8O_4$ (found: C, 53.94; H, 5.16. Calc. for $C_7H_8O_4$: C, 53.85; H, 5.16%). UV- and IR-spectral properties were λ_{max} (EtOH) 267 nm (ε 7960); ν_{max} ($CHCl_3$) 3510 (OH), bands between 3000 and 2400 (COOH), 1718 (COOH dimer), 1672 (conjugated C=O), 1621 (C=C) cm^{-1}. The 100 MHz NMR-spectrum (in $CDCl_3$) consisted of signals at τ 1.53 (singlet, 1P, disappears on addition of D_2O; OH), 2.63 (singlet, 1P; CO.C=CH.O), 5.48 (triplet, 2P, J=7Hz; CH_2O), 6.83 (singlet, 2P; CO · CH_2 · C = C), and 7.34 (triplet, 2P, J = 7Hz; CO · CH_2); each triplet collapsed to a singlet on irradiation at the frequency of the other triplet. The mass spectrum (70 e.v.) showed a parent ion at *m/e* 156 and other prominent ions at *m/e* 112 ($M-CO_2$), 83, 60 [$CH_2 = C(OH)_2^+$], 55, 39 and 27[6]. Consideration of the foregoing evidence led to formulation of the metabolite as 2, 3-dihydro-4-pyrone-5-acetic acid (II), desoxypatulinic acid [m.p. 114.5–115.5 °C; λ_{max} (EtOH) 268 nm][1,2]. This assignment was confirmed by direct comparison of the metabolite (mixed m.p., TLC, and IR-, UV- and mass spectra) with desoxypatulinic acid, m.p. 113.5–114.5 °C, prepared by hydrogenolysis of chlorodesoxypatulinic acid[2]. Desoxypatulinic acid (46 µg/ml tryptic soy agar) had no inhibitory effect on *Bacillus megaterium, B. subtilis, B. cereus, Staphylococcus aureus, S. epidermidis, Sarcina lutea, Micrococcus flavus*, and *Saccharomyces cerevisiae* (cf. patulin was completely inhibitory under the same conditions).



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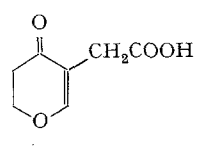

II

Desoxypatulinic acid was readily detected by TLC on silica gel F-254 (0.25 mm) developed with toluene-ethyl acetate-formic acid (6:3:1, v/v) as a dark spot under short wavelength UV-light at Rf 0.22. Analysis by TLC of aliquots of culture medium withdrawn during the fermentation showed that desoxypatulinic acid and patulin (Rf 0.33) concentrations reached a peak at about the same time (10–12 days); patulin was no longer detectable on day 17 although traces of the acid remained. The relationship of desoxypatulinic acid to the pathway of patulin biosynthesis[4,7,8] merits investigation.

Zusammenfassung. Aus Kulturlösungen von *Penicillium patulum* Bainier wurde neben Patulin die Desoxypatulinsäure isoliert und identifiziert.

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Systematics of a Leptospira Strain Isolated from Frog

In the year 1964, in Iowa, USA, DIESCH et al.[1,2] isolated a leptospira strain from a pool of kidneys of 3 specimen of frogs (*Rana pipiens*) collected during follow-up of a human leptospirosis outbreak associated with swimming.

This is the first case reported in the literature of isolation of a leptospira strain from the organs of an amphibian. Therefore, it is of particular interest, also for epidemiological purposes, to ascertain whether this strain belongs to pathogenic leptospiras (*L. interrogans*) or to saprophytic ones ('biflexa' complex), and whether this strain might be inserted in the group of leptospiral serotypes that we know already. DIESCH et al.[3] have executed some surveys in this direction without being able to attain a sure conclusion. In fact, the leptospira, inoculated into guinea-

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