1,4-Dihydroxy-2-methoxy-6-methylbenzene, a Metabolite of *Penicillium baarnense*

JULIAN BETTER and STEN GATENBECK

Department of Pure and Applied Biochemistry, The Royal Institute of Technology, S-100 44 Stockholm, Sweden

The mould *Penicillium baarnense* produces as secondary metabolites among other substances orsellinic acid, penicillic acid and barnol.[1] The relative amounts of these substances are highly dependent on the composition of the culture substrate. When Czapek-Dox medium is used, only trace amounts of barnol are formed whereas orsellinic acid and penicillic acid are produced in substantial amounts. The reverse situation is at hand, when the organism is grown on Raulin-Thom medium. When investigating this phenomenon the formation of a phenolic compound was observed irrespective of culture substrate used. The phenolic compound appeared as the first phenolic substance excreted into the culture fluid when the organism was grown on Raulin-Thom medium. However, the compound was reutilized by the organism at the time of barnol production. As the compound could be on the pathway of barnol synthesis it seemed important to establish its structure.

The organism was grown on Czapek-Dox medium in a fermentation tank. The phenolic compound was isolated from the culture filtrate by extraction with organic solvent. The purification procedure involved washing with aqueous hydrogen carbonate, sublimation and recrystallization from carbon tetrachloride, m.p. 130–131 °C, ν_{max} (KBr) 3320, 1610 cm^{-1}, λ_{max}(MeOH) 208 (12 660), 288.5 (3918) nm. The NMR spectrum ($CDCl_3$) showed resonances due to one aromatic methyl group (δ 2.12, 3 H, s), one methoxy group (δ 6.10, 3 H, s) and the signals of two aromatic protons (δ 6.36 dd, J 2.0 Hz). Resonance that could be assigned to two phenolic hydroxy groups (δ 5.27, 2 H, s) were also visible. The mass spectrum had a large molecular ion peak (% relative intensities), *m/e* 154 (100) and further abundant fragments were found at *m/e* 139 (79) and *m/e* 111 (51).

The spectroscopic data indicated the structure shown in the figure. This structure was confirmed by comparison with a synthetic specimen. The preparation of 1,4-dihydroxy-2-methoxy-6-methylbenzene was performed by reduction with sodium dithionite of the corresponding quinone, the synthesis of which was recently reported.[2] It has been demonstrated that the quinone is an intermediate in the biosynthesis of penicillic acid in *Penicillium cyclopium*.[2,3] The presence of its dihydro derivative in *P. baarnense* has presumably no relation to the barnol formation but to the penicillic acid synthesis in this organism. Apparently, when *P. baarnense* is grown on Raulin-Thom medium the organism develops an alternative route for the degradation of 1,4-dihydroxy-2-methoxy-6-methylbenzene which does not lead to penicillic acid.

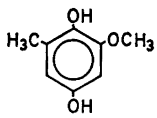

Fig. 1. 1,4-Dihydroxy-2-methoxy-6-methylbenzene.

Experimental. Infrared spectra of KBr discs were recorded on a Perkin-Elmer model 257 spectrometer and ultraviolet spectra on a Beckman DK-2. The NMR spectra ($CDCl_3$) were recorded on a Varian Anaspect EM 360 instrument with TMS as internal standard and the mass spectrum on a LKB instrument type 9000 using the direct inlet system.

Penicillium baarnense v. Bayma, CBS 315.59 was grown in 5 l of Czapek-Dox medium ($NaNO_3$, 2.0 g; KH_2PO_4, 1.0 g; KCl, 0.5 g; $MgSO_4.7H_2O$, 0.5 g; $FeSO_4.7H_2O$, 0.01 g; yeast extract, 1.0 g; glucose, 40 g; distilled water to 1000 ml) using a New Brunswick tank fermentor (300 rpm, aeration 4000 ml/min) at 27 °C. The fermentor was inoculated with culture from two 500 ml shake flasks pregrown for 3 days on the same medium. After 4 days the mycelium was filtered off and washed with water. After acidification with HCl the filtrate was extracted with ether. The acid substances were removed from the ether phase by washing with aqueous $NaHCO_3$ followed by distilled water. The ether solution was dried over anhydrous Na_2SO_4 and then evaporated to dryness in a rotary evaporator. The semisolid residue was sublimed at 12 mmHg and 100 °C and the white sublimate repeatedly recrystallized from CCl_4, yield 100 mg.

Acknowledgement. This work was supported by grants from the Swedish Natural Science Research Council.

1. Mosbach, K. and Ljungcrantz, I. *Physiol. Plant. 18* (1965) 1.
2. Axberg, K. and Gatenbeck, S. *Acta Chem. Scand. B 29* (1975) 749.
3. Axberg, K. and Gatenbeck, S. *FEBS Lett. 54* (1975) 18.

Received January 12, 1976.