LETTER TO THE EDITOR

TRANSFORMATION OF 3-HYDROXYPYRIDINE BY *Pseudomonas fluorescens* AND *RHODOCOCCUS OPACUS* STRAINS

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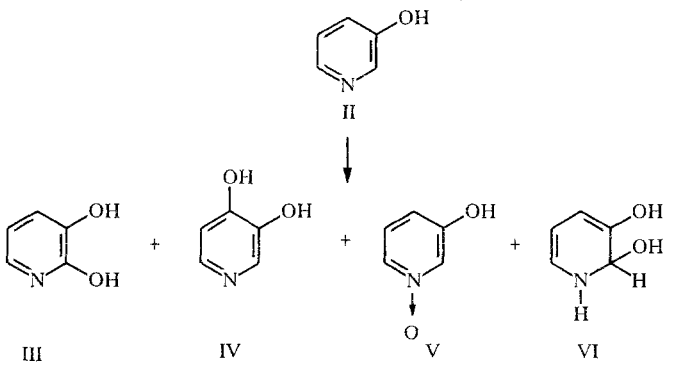
The initial step in the catabolism of benzene by microorganisms of the *Pseudomonas* genus is its hydroxylation to cis-1,2-dihydroxy-3,5-cyclohexadiene [benzenecisglycol (I)]. Other aromatic compounds (toluene, naphthalene, biphenyl, etc.) are also oxidized similarly by this culture. A biotechnological method for the preparative synthesis of glycol I and its analogs; the promising character of their use in the synthesis of a number biologically active natural compounds has been demonstrated [1]. Microorganisms that hydroxylate aromatic compounds can be successfully used to obtain a whole host of valuable biologically active compounds [2, 3]. From a practical point of view, virtually no research has been devoted to heteroaromatic compounds.

We have shown that *Pseudomonas fluorescens* PfE1 bacteria, which utilize naphthalene, oxidize unsubstituted pyridine only at the nitrogen atom. In a neutral medium 3-hydroxypyridine (II) is converted completely by this culture to a mixture consisting of 2,3- and 3,4-dihydroxypyridines III and IV, 3-hydroxypyridine N-oxide (V), and 1,2-dihydro-2,3-dihydroxypyridine (VI) in approximately equal amounts.



Substrate II is oxidized at pH 5-6 to N-oxide V, while only diols III and IV are formed in an overall yield of 19% in an alkaline medium. It is likely that oxidation at the nitrogen atom and hydroxylation of the heteroring are realized by different enzyme systems.

The root-form *Rhodococcus opacus* (the M form) bacteria, which utilize pyridine, do not, in contrast to *Ps. fluorescens*, form N-oxide V but rather convert II to a mixture of diols III and IV in 12% and 32% yields, respectively.

The transformation reaction is realized by suspension of the nonreplicating cells in a 0.1 M phosphate buffer at 28°C for 48 h (starting concentration of the substrate was 250 mg/liter). The cells were separated by means of a centrifuge, and the supernatant was extracted with chloroform. The chloroform and the aqueous residue were evaporated in vacuo, and the products were isolated by chromatography in a thin layer of silica gel in a petroleum ether–ethyl acetate–ethanol (10:10:2) system. The individual compounds were analyzed by the UV and mass-spectral data.

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2,3-Dihydroxypyridine (III, $C_5H_5NO_2$). This compound had R_f 0.13, and the respective yields were as follows: 9% (*Ps. flluorescens)* and 12% (*Rh. opacus*). UV spectrum (ethanol): λ_{max} 297 nm (297 nm [4]). Mass spectrum, m/z (intensity, %): 111 (100, M), 94 (7), 84 (6), 83 (20), 82 (20), 68 (5), 56 (15), 55 (16), 54 (17).

3,4-Dihydroxypyridine (IV, $C_5H_5NO_2$). This compound had R_f 0.11. The yields were as follows: 10% (*Ps. fluorescens*) and 32% (*Rh. opacus*). UV spectrum (water): λ_{max} 273 nm (273 nm [4]). Mass spectrum, m/z (intensity, %): 111 (100, M), 94 (23), 84 (5), 55 (41), 54 (7).

3-Hydroxypyridine N-Oxide (V, $C_5H_5NO_2$). This compound had R_f 0.32 and was obtained in 7% yield (*Ps. fluorescens*). UV spectrum (ethanol): λ_{max} 262 nm. Mass spectrum, m/z, (intensity, %).: 111 (100, M), 95 (17), 83 (27), 68 (6), 67 (8), 66 (5).

1,2-Dihydro-2,3-dihydroxypyridine (VI, $C_5H_7NO_2$). This compound was obtained in an 11% yield (*Ps. fluorescens*). Mass spectrum, m/z (intensity, %): 113 (79, M), 112 (57), 95 (14), 84 (21), 83 (43), 70 (100), 68 (7), 55 (64).

1,2-Dihydro-2,3-bis(trifluoroacetoxy)pyridine ($C_9H_5F_6NO_4$). This compound was obtained by treatment of VI with bis(trifluoroacetyl)methylamine. Mass spectrum, m/z (intensity, %): 305 (8, M), 277 (10), 249 (23), 191 (9), 163 (7), 151 (18), 135 (7), 122 (7), 95 (16), 69 (100).

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