Preparation of 6-Oxo-1,6-dihydropyridine-2-carboxylic Acid by Microbial Hydroxylation of Pyridine-2-carboxylic Acid

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Alcaligenes faecalis (DSM 6269) grown on pyridine-2-carboxylic acid induced regiospecific hydroxylation of the latter to 6-oxo-1,6-dihydropyridine-2-carboxylic acid on a preparative scale.

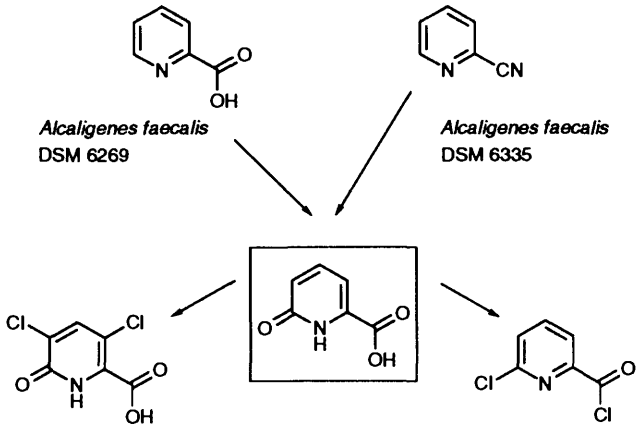
Substituted pyridine-2-carboxylic acids (picolinic acids) are important intermediates for the preparation of pharmaceuticals and agrochemicals.[1,2] However, the regiospecific functionalization of picolinic acid is difficult to achieve by chemical methods. For example, although 6-oxo-1,6-dihydropyridine-2-carboxylic acid (6-hydroxypicolinic acid) can be prepared in 51% yield by treatment of aqueous potassium picolinate with elemental fluorine,[3] the industrial applicability of such a reaction is hampered by both its poor selectivity and the disposal costs of the chemical waste. Here we describe a simple and ecologically advantageous method for the large-scale preparation of 6-hydroxypicolinic acid by regiospecific microbial hydroxylation of picolinic acid.[4]

The microbial degradation of picolinic acid, nicotinic acid and isonicotinic acid proceeds *via* the initial formation of 6-hydroxypicolinic acid, 6-hydroxynicotinic acid and 2-hydroxyisonicotinic acid, respectively.[5] In the microbial degradation of nicotinic acid the presence of high nicotinic acid concentrations prevents the further conversion of 6-hydroxynicotinic acid into the subsequent metabolite pyridine-2,5-diol. Wild type strains of nicotinic acid-degrading bacteria are, therefore, able to produce > 100 g dm^{-3} of 6-hydroxynicotinic acid in excellent yield from nicotinic acid.[6]

We have observed that the wild type microorganism *Alcaligenes faecalis* DSM 6269, growing at the expense of picolinic acid, showed an analogous regulation of the picolinic acid degradative pathway as mentioned above for the nicotinic acid-degrading organism. This bacterium accumulated in the presence of picolinic acid concentrations > 2 g dm^{-3} of 6-hydroxypicolinic acid; further metabolism of 6-hydroxypicolinic acid occurred only when all picolinic acid was hydroxylated. Picolinic acid esters, isonicotinic acid and methylpyridines were no substrates for the hydroxylation.

Following the simple fermentation procedure outlined below we were able to produce 98 g dm^{-3} of 6-hydroxypicolinic acid within a fermentation time of 42 h: *Alcaligenes faecalis* DSM 6269 was maintained on Petri dishes with a mineral salts medium[7] containing sodium picolinate (1.2 g dm^{-3}) as sole carbon source and agar (16 g dm^{-3}). The incubation temperature was 30 °C. Precultures were grown on mineral salts medium supplemented with 2.4 g dm^{-3} of sodium picolinate. A fermenter (23 dm^3) containing mineral salts medium (15 dm^3) supplemented with sodium picolinate (1.2 g dm^{-3}), pH 7.0, was used for preparative biotransformations. The concentration of picolinic acid and 6-hydroxypicolinic acid during the biotransformation was followed spectrophotometrically. A solution containing picolinic acid (500 g dm^{-3}) was used as acid for pH control. After 27 h of growth 2 dm^3 of the picolinic acid solution had been consumed and the resulting biomass had accumulated 20 g dm^{-3} of 6-hydroxypicolinic acid. At this time the oxygen partial pressure of the cell suspension was 1% of the maximal saturation (agitation speed 750 rev. min^{-1}; aeration rate 30 dm^3 min^{-1}). To increase the product concentration 2.5 dm^3 of a solution containing sodium picolinate (470 g dm^{-3}) was pumped over a period of 15 h into the cell suspension. When the concentration of 6-hydroxypicolinic acid ceased to increase the cells were removed by centrifugation. 6-Hydroxypicolinic acid was precipitated by acidifying the cell-free fermentation solution with sulfuric acid to pH 1.5. A total of 2190 g of picolinic acid were used for this batch and 1850 g were isolated as 6-hydroxypicolinic acid. The overall yield was 75%, including the picolinic acid required for the production of the biomass. The purity of the isolated material was > 95% as judged by HPLC analysis. The solubility of 6-hydroxypicolinic acid in water at 25 °C was 3.1 g dm^{-3} compared to > 500 g dm^{-3} for picolinic acid.

The efficient biotransformation of picolinic acid into 6-hydroxypicolinic acid opens a new route for the preparation of 6-substituted or 3,5,6-trisubstituted picolinic acid derivatives as outlined in Scheme 1.



Scheme 1

A second microorganism *Alcaligenes faecalis* DSM 6335, growing at the expense of 2-cyanopyridine, also accumulated 6-hydroxypicolinic acid in biotransformations using 2-cyanopyridine as a substrate. The biocatalyst was grown using a similar protocol as described above for *A. faecalis* DSM 6269 in which the picolinic acid was substituted for 2-cyanopyridine. With *A. faecalis* DSM 6335 we were able to produce 5.5 g dm^3 6-hydroxypicolinic acid with a 40% yield from 2-cyanopyridine.

We are currently optimizing both the fermentation and biotransformation conditions for an industrial application of these bioconversions.

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References

1 C. J. Foster, T. Gilkerson and R. Stocker, European Patent # 447,004; (*Chem. Abstr.*, 1992, **116**, 128668e).
2 Y. Ito, H. Kato, E. Etsuchu, K. Mitani, N. Yagi, T. Yoshida and N. Iwasaki, Japanese Patent # 63,150,277 (*Chem. Abstr.*, 1989, **110**, 23895z).
3 M. Van Der Puy, D. Nalewajek and G. E. Wicks, *Tetrahedron Lett.*, 1988, **29**, 4389.
4 This work was previously published in European Patent # 498 316 and European Patent # 504 818.
5 Review article: O. P. Shukla, *J. Sci. Ind. Res.*, 1984, **43**, 98.
6 H. G. Kulla, *Chimia*, 1991, **45**, 81.
7 H. G. Kulla, F. Klausener, U. Meyer, B. Lüdeke and T. Leisinger, *Arch. Microbiol.*, 1983, **135**, 1.

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