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BACTERIA IN ORGANIC SYNTHESIS: SELECTIVE CONVERSION OF 1,3-DICYANOBENZENE INTO 3-CYANOBENZOIC ACID

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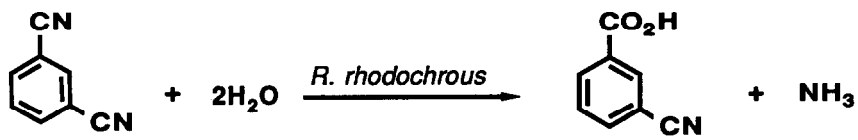
Summary: Suspensions of Rhodococcus rhodochrous NCIB 11,216 catalyse hydrolysis of dinitriles into cyanocarboxylic acids under mild conditions. This bioconversion was used for the highly selective synthesis of 3-cyanobenzoic acid from 1,3-dicyanobenzene.

Acid catalysed hydrolysis of nitriles usually requires prolonged boiling with a concentrated solution of acid and therefore cannot be applied to acid sensitive or epimerisable molecules. Although recently milder catalytical procedures for nitrile hydrolysis were developed,[1] they are complex and require onerous isolation and purification steps. Furthermore, by chemical means, it is virtually impossible to selectively convert one cyanogroup of a polynitrile. Biological hydrolysis of nitriles[2] offers an interesting alternative as it can be performed at moderate temperatures and at pH values close to neutrality. Several researchers reported bacterial hydrolysis of aliphatic[3] and aromatic[4] nitriles into corresponding carboxylic acids or amides, however the processes are not adapted to selective conversion of polynitriles and very little is apparently known about the applicability of such a technique for practical synthetic purposes.

We observed that resting cells of Rhodococcus rhodochrous NCIB 11,216, which were previously grown on benzonitrile, selectively hydrolysed aromatic dinitriles into correpsonding cyanocarboxylic acids. We now report the first results as applied to the preparation of 3-cyanobenzoic acid from 1,3-dicyanobenzene in nearly quantitative yield.



The following experiment is representative: Cells of R. rhodochrous (maintained on 1.5% w/v agar, containing per litre 10 g triptone, 5 g yeast extract and 5 g NaCl), were grown on the pH 7 basal salts medium containing per litre: 6 g Na_2HPO_4, 3 g KH_2PO_4, 0.5 g NaCl, 0.2 g $MgSO_4 . 7H_2O$ and 5 ml trace metals solution[5] with 10 mM benzonitrile as the sole carbon and nitrogen source. Cultures were grown aerobically at 28 °C in 2 l flasks containing 750

ml medium on a reciprocal shaker at 120 rpm and bacterial growth was monitored by measuring the optical density at 690 nm. Cells were harvested by centrifugation after 20 hr, in the early-mid exponential stage (1.2-1.7 OD) and washed with 0.1 M phosphate buffer at pH 7. The cells (2 g wet weight) were then resuspended in 0.25 M phosphate buffer pH 7 and incubated on a shaker with 0.57 g (4.45 mmol) of 1,3-dicyanobenzene at 30 ^{o}C for 25 hr. Aliquots were withdrawn periodically and reaction progress was monitored by quantitative determination of ammonia released into solution[6]. Upon release of 1 eq. of ammonia the reaction came to a standstill. Since the cells were shown to be active when incubated with a fresh batch of the dinitrile, evidently the bioconversion stopped at the stage of monohydrolysis. Acidification of the supernatant followed by extraction with chloroform afforded pure 3-cyanobenzoic acid in 95% yield[7].

The kinetics of ammonia release indicated that the benzonitrile grown cells are similarly effective in half hydrolysis of 1,4-dicyanobenzene to form the corresponding 4-cyanobenzoic acid. However, when these cells were incubated with aliphatic dinitriles such as: $NC-(CH_2)_n-CN$ (n=3,4,5), hydrolysis proceeded at a very low rate and was not selective. On the other hand, resting cells of the bacterium, which were previously grown on propionitrile, had a remarkably different substrate affinity, and hydrolysed effectively (although not selectively) the above mentioned aliphatic dinitriles rather than the aromatic.

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7. Spectroscopic data identical to those of an authentic sample.

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