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Microbial Synthesis of Pyrrole-2-Carboxylate by *Bacillus megaterium* PYR2910

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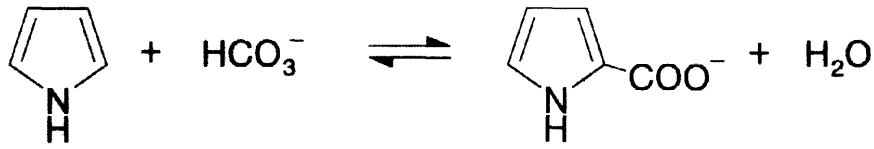
Abstract: Pyrrole-2-carboxylate was synthesized from pyrrole using the carboxylation reaction of reversible pyrrole-2-carboxylate decarboxylase from *Bacillus megaterium* PYR2910. By addition of high amounts of bicarbonate, the reaction equilibrium was shifted towards pyrrole-2-carboxylate.

Keywords: Carboxylation; Enzymes and enzyme reactions; Pyrroles

C-C bond forming enzymes are of interest for preparative organic chemistry. Pyrrole-2-caboxylate decarboxylase from *Bacillus megaterium* PYR2910 catalyzes the decarboxylation of pyrrole-2-carboxylate to stoichiometric amounts of pyrrole and bicarbonate (HCO_3^-) [1]. The enzyme was found also to catalyze the reverse carboxylation of pyrrole (**1**) to pyrrole-2-carboxylate (**2**) after addition of HCO_3^- [2]:



1 **2**

The concentration of solubilized HCO_3^- was the carboxylation limiting factor. Therefore, saturating amounts of $KHCO_3$ (3 M) were used leading to a shift of the reaction equilibrium towards the carboxylate. HCO_3^- addition was accompanied by CO_2 gas evolution resulting in an increased pressure in the tightly closed reaction vessel determined to 1.38 atmosphere by a connected pressure gauge. The increased pressure supported the reverse reaction productivity 2.5-fold compared to atmospheric pressure. High pressures are also applied in organic synthesis carboxylations [3]. As biocatalyst, concentrated cells with an optical density at 610 nm of 40, previously grown under inducing conditions [1], were employed. Additionally, acetate as enzyme cofactor and L-ascorbate as antioxidizing,

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enzyme protecting agent [1] were added to reaction mixture. As a result of carbon and oxygen incorporation, 26.1 g/l (235 mM) pyrrole-2-carboxylate were formed from 20.7 g/l (300 mM) pyrrole. The yield after bioconversion was 80%, limited by the equilibrium.

The product was isolated by centrifugation of the reaction mixture (40000x*g*, 30 min) and applying the supernatant to a Dowex-1 (Dow Chemicals, USA) anion exchange chromatography previously equilibrated with water. Water was used to wash out pyrrole. The product was eluted with 2 M acetic acid, concentrated in a vacuum evaporator and crystallized in H_2O. The crystals were identified as pyrrole-2-carboxylate by ^1H NMR (Joel JNM-400 FT NMR, 400 MHz, DMSO) and infrared spectroscopy (Perkin Elmer 1600 FTIR) using authentic pyrrole-2-carboxylate as reference. The overall yield after isolation was 52%.

Pyrrole-2-carboxylate is employed in the synthesis of various pharmaceuticals [4,5] and a potential herbicide [6]. A number of organic syntheses were described including the reaction of pyrrolylmagnesium bromide with powdered solid CO_2 [7], the treatment of 1,2 oxazines, obtained by reacting butadienecarboxylic esters with nitrosobenzene, with a base in ethanol [8], the addition of toluene-*p*-sulphonylglycine to α,β-unsaturated ketones [9] and the formylation of pyrrole to the 2-aldehyde and further oxidation with alkaline silver oxide [10]. However, these syntheses require multiple steps and result in low yields [10]. Furthermore, the chemical carbonation of pyrrole with K_2CO_3 requires high pressure and temperature [11]. The one-step bioconversion described here has advantages with regard to regiospecificity, yield and mild reaction conditions. Unfortunately, the enzyme is highly specific for pyrrole, and a number of pyrrole analogs were not carboxylated.

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