NONENZYMIC DECARBOXYLATION OF THE AMINOBENZOATES*

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The decarboxylation of p-aminobenzoate and anthranilic acid to aniline by cell free preparations from Escherichia coli has been reported by McCullough et al. (McCullough et al., 1956). The enzyme is unable to decarboxylate the meta isomer of the aminobenzoate. Furthermore, it has been established that the reaction requires pyridoxal phosphate as a cofactor. It has been proposed by Kosower (Kosower, 1962) that an additional coenzyme is required along with B_6 and acts in a reversible fashion as an electron source. However, there is no experimental evidence to substantiate this proposal.

We have been able to duplicate these reactions nonenzymatically using a model system in which only pyridoxal served as the catalyst. No other electron source was required for these decarboxylations. The results of refluxing a mixture of pyridoxal and the separate aminobenzoates are given in Table I. Decarboxylation of the para isomer proceeded in a linear fashion over a three hour period (Table I). Aniline production from the ortho isomer proceeds at a rate which is 16 times as fast as that observed for the para isomer of amino benzoate under similar experimental conditions. These results are compatible with those reported for the enzymatic reaction in which the ortho isomer is decarboxylated at a much higher rate than the para isomer (MCullough et al., 1956). The meta isomer is inactive in both the enzymic (McCullough et al., 1956) and nonenzymic systems.

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Table I

Reaction of o-, m- and p-aminobenzoates in the presence of pyridoxal

The complete reaction mixture contained 600μ moles of pyridoxal, 585μ moles of aminobenzoate in 23 ml of solution buffered at pH 7.2 with 0.5M pH 7.2 phosphate buffer.(1)

Time (hr.)	Pyridoxal	p-Aminobenzoate	m-Aminobenzoate	o-Aminobenzoate	μMoles Aniline(2)
1	X	X	0	0	0.37
2	X	X	0	0	1.0
3	X	X	0	0	1.5
3	X	0	0	X	24.0
3	X	0	X	0	0

(1) When pyridoxal was omitted, no aniline was formed.

(2) The aniline was determined, after extraction with petroleum ether, by the method of Bratton and Marshall (Bratton and Marshall, 1939).

The results in Table I were obtained by reading the experimental samples against blanks consisting of aminobenzoate and buffer heated for the same periods This permitted the correction for the small spontaneous decarboxylation usually observed. Significant productions of aniline were observed in the presence of but not the absence of pyridoxal. The results in Table I demonstrate that the decarboxylation reaction is pyridoxal-dependent.

The decarboxylation of the aminobenzoate is unique since it does not fit the general mechanism of B_6 catalyzed reactions (Metzler et al., 1954). An examinati of the structural formula of the Schiff base of pyridoxal and the aminobenzoates shows that decarboxylation could not occur through a withdrawal of electrons from the bond to the carbonyl group in the manner suggested for other B_6 catalyzed reactions (Metzler et al., 1954).

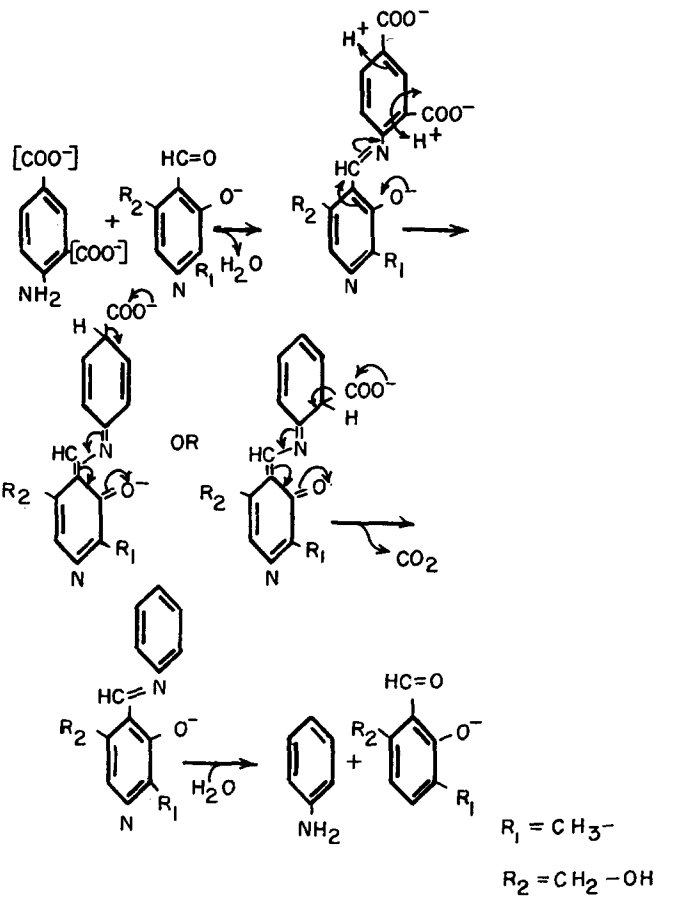

Fig. 1 - Diagrammatic representation of the decarboxylation of para and ortho aminobenzoates in the presence of pyridoxal.

However, as shown in Fig. 1, decarboxylation could occur by means of an addition to a proton followed by loss of $-CO_2-$. The role of the pyridoxal as a catalyst in this reaction would be to supply electrons at the ortho and para positions of the aminobenzoates. The only group on the pyridoxal capable of supplying electron in this fashion is the phenolic group. By an electromeric mechanism of electron release, as shown in Fig. 1, the electrons are mobilized and supplied at the ortho and para positions from the phenolic group. Decarboxylation then results with a movement of the electrons back on to the phenolic oxygen of pyridoxal. Subsequent hydrolysis or displacement by another molecule of the substrate results in the release of aniline.

The decarboxylation was carried out at pH 7.2 because slightly alkaline conditions should favor the reaction. That is, under conditions of sufficiently high alkalinity that the acid is present as an anion. However, the basicity of the reaction mixture should be low enough so that the concentration of hydrogen ions is not too greatly depressed.

REFERENCES

1. McCullough, W. G., Pelligian, J. T. and Daniel, I. J., J. Am. Chem. Soc. 79, 628 (1956)
2. Kosower, E. M., "Molecular Biochemistry", McGraw-Hill, Inc., New York, N.Y., 1963, p. 124
3. Bratton, A. C. and Marshall, E. K., J. Biol. Chem. 128, 537 (1939)
4. Metzler, D. E., Ikawa, M. and Snell, E. E., J. Am. Chem. Soc. 76, 648 (1954) ibid. 76, 650 (1954)