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THE BIOSYNTHESIS OF PHENAZINES: INCORPORATION OF PHENAZINE-1,6-DICARBOXYLIC ACID IN ETHER-TREATED CELLS OF PSEUDOMONAS AUREOFACIENS

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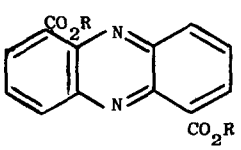
Summary: Ether treatment of Pseudomonas aureofaciens cells facilitates assimilation of phenazine-1,6-dicarboxylic acid (1) and (1) is shown to be an efficient and specific precursor for phenazine-1-carboxylic acid (3).

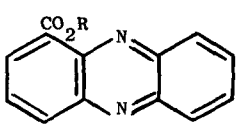
Phenazine-1,6-dicarboxylic acid (1) is logically the first of the microbial phenazines to be formed, the one from which all the others derive. However, whilst phenazine-1,6-dicarboxylic acid (1) is a precursor for phenazines produced by some Actinomycetes,[1,2] it fails to be incorporated into phenazines produced by Pseudomonas and related species.[2,3] Negative results with the latter group of bacteria may be attributed to poor transport of (1) across the cell wall to the site of phenazine biosynthesis. Bacterial cell walls, including those of Pseudomonas species, have been made permeable to peptido-glycan and DNA precursors not normally assimilated by treatment of the cells with toluene or diethyl ether.[4] We have applied this technique to the study of phenazine biosynthesis.

P. aureofaciens produces phenazine-1-carboxylic acid (3) and 2-hydroxyphenazine-1-carboxylic acid (5). Cells of this organism, isolated from shake cultures at the beginning of phenazine production, were shaken in Ringers solution-Tris buffer, pH7, with diethyl ether at 9° for 10 minutes, and were then reintroduced into the original medium. These cells efficiently incorporated [dicarbonyl-$^{14}C_2$]phenazine-1,6-dicarboxylic acid [as (1)] into (3) (2.6% incorporation) and also to some extent into (5) (0.2% incorporation); the incorporation of (1) into (3) was shown to be specific by decarboxylation of the labelled (3) which gave inactive phenazine.

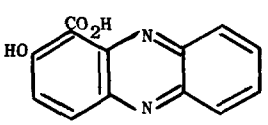
Using non-ether treated cells (1) was also transformed into (3), but at a lower level (1.2%). A similar result was obtained with undisturbed cultures (1.0% incorporation). This was



(1) R = H
(2) R = Me



(3) R = H
(4) R = Me



(5)

surprising in view of previous negative results,[2,3] but we noted, tentatively, that cells that were producing phenazines at a high rate gave, in all cases, higher incorporations. It is clear that under the right conditions (1) can act as a precursor for (3) and (5), and its incorporation is markedly enhanced by using ether-treated cells. We conclude on the basis of these results and of others[1,2] that phenazine-1,6-dicarboxylic acid (1) is a universal and key intermediate in phenazine biosynthesis.

Using ether-treated cells, we have found, in confirmation of earlier results,[1,2] that dimethyl phenazine 1,6-dicarboxylate (2) is a very poor precursor for (3) and (5). On the other hand, methyl [carbonyl-^{14}C]phenazine-1-carboxylate [as (4)] was transformed into (3) with high efficiency (16% and 14% incorporation in two experiments). A hydrolase is thus present which is active with the ester (4), but not (2), as substrate. Labelled methyl phenazine-1-carboxylate (4) was also transformed into (3) in untreated cells but, as with (1), the efficiency of the transformation was markedly reduced (4% incorporation). The marked enhancement of substrate assimilation in ether-treated cells of P. aureofaciens suggests application of this technique in the study of the biosynthesis of other secondary metabolites.

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