FORMATION OF RESORCINOL AS AN INTERMEDIATE IN THE METABOLISM OF 2,4-DIHYDROXYBENZOIC ACID AND ITS CORRESPONDING ALDEHYDE*

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Numerous reports have appeared concerning the mechanisms by which simple benzene-ringed compounds are modified prior to the rupturing of the aromatic ring. Evans and Ribbons (1950) have presented a concise summary of studies in this area. In all non-nitrogenous compounds, rupture occurs only provided hydroxyl groups are present at two sites in the ring. Where these hydroxyl groups are ortho to one another, such as in the case of catechol and protocatechuic acid, ring cleavage may occur either across the hydroxyl groups or adjacent to one of the hydroxyl groups. Where the hydroxyl groups are para to one another, such as in the case of gentisic acid and homogentisic acid, ring rupture occurs between the side chain and the o-hydroxyl group without further modification of the side group. In no case has a meta-hydroxylated compound been involved in direct ring rupture.

An Aspergillus sp. has been isolated which is able to use 2,4-dihydroxybenzoic acid as a sole source of carbon in a

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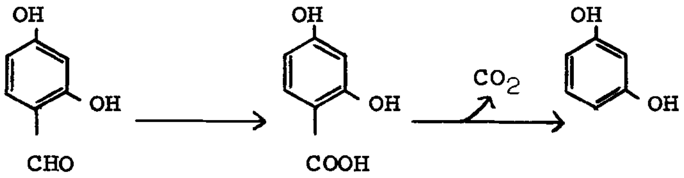
basal mineral salts medium. Practical grade 2,4-dihydroxybenzoic acid was recrystallized in methanol-water until chromatographically pure and used at a level of 10 µmoles/ml (~0.20%) in mineral salts solution of the following composition: KH_2PO_4, 1 gm/l.; K_2HPO_4, 1 gm/l.; NH_4NO_3, 1 gm/l.; $MgSO_4.7H_2O$, 0.02 gms/l.; $CaCl_2.2H_2O$, 0.02 gms/l.; and $FeCl_3.6H_2O$, 0.003 gms/l. The rate of 2,4-dihydroxybenzoic acid degradation was followed by paper chromatography using diazotized sulfanilic acid (Bray, Thorpe and White, 1950) as the detecting reagent followed by an overspray of 20% Na_2CO_3. 2,4-Dihydroxybenzoic acid disappeared completely from the medium upon incubation. A second spot appearing in fermentation liquor was identified as resorcinol on the basis of Rf values in several solvent systems and comparison of ultra-violet absorption spectrum with that of authentic sample. In addition the spot disappeared from the fermentation liquor sooner than did 2,4-dihydroxybenzoic acid. As well, resorcinol served not only as a sole carbon source in the same mineral salts mixture, but was metabolized at a faster rate than 2,4-dihydroxybenzoic acid; an equi-molar quantity disappeared in 12 days as compared to 17 days for 2,4-dihydroxybenzoic acid.

A normal pattern for the modification of an aromatic side chain constitutes oxidation through the corresponding alcohol, aldehyde, and then to the acid, e.g., p-cresol ⟶ p-hydroxybenzyl alcohol ⟶ p-hydroxybenzaldehyde ⟶ p-hydroxybenzoic acid (Dagley and Patel, 1957). 2,4-Dihydroxybenzaldehyde failed to serve as a substrate for growth. But when mycelial mats prepared by growing the Aspergillus sp. on nutrient broth containing added glucose (1.0%) were floated over 2,4-dihydroxybenzaldehyde (10 µmoles/ml) in the absence of mineral salts, the corresponding acid was readily identified on chromatograms and subsequently was metabolized. Under these conditions, resorcinol did not accumulate in sufficient amounts to be detected.

The successive appearance and disappearance of identified products coupled with a satisfactory growth response is ample evidence to establish the following mechanism:



2,4-Dihydroxybenzaldehyde
2,4-Dihydroxybenzoic acid
Resorcinol

Such a mechanism is most interesting in that it differs from the metabolism of gentisic acid (2,5-dihydroxybenzoic acid) which undergoes direct ring rupture rather than a decarboxylation process (Lack, 1959). This suggests that the positions of the hydroxyl group may have a regulating effect upon the pathway through which degradation proceeds. Investigations are now being made on the exact mechanism of resorcinol disappearance.

References

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