

TITLE: Biotic and Abiotic Carbon To Sulfur Bond Cleavage

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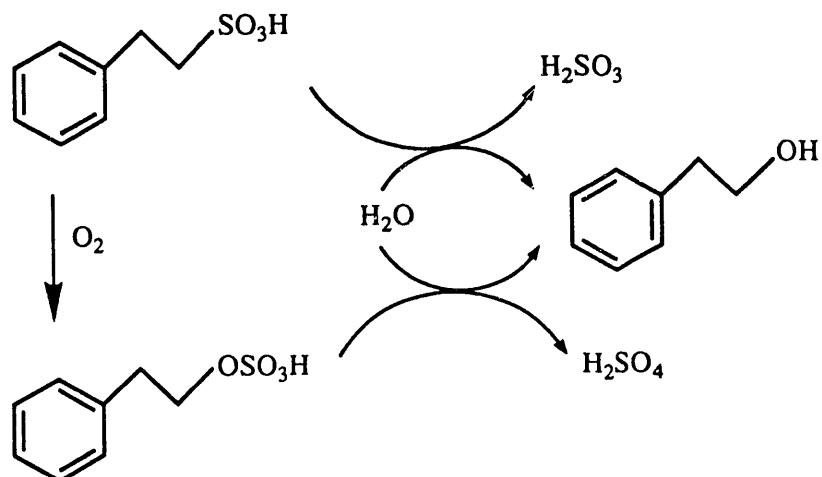
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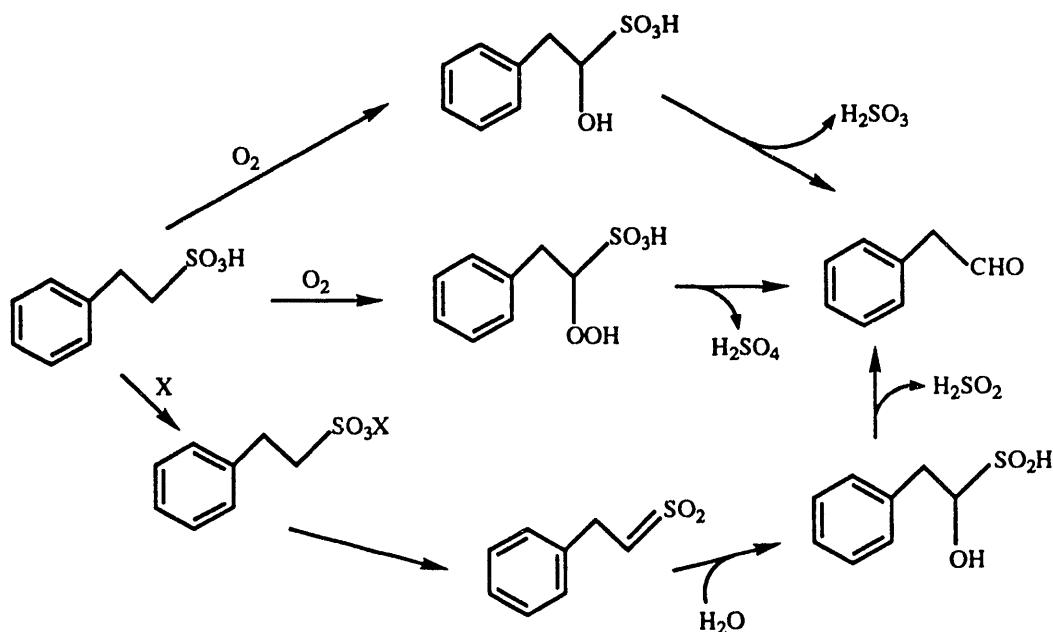
Mechanisms for cleavage of $\text{RCH}_2\text{-S}$ bonds catalyzed by *Escherichia coli* can best be categorized by whether an alcohol RCH_2OH (Scheme I) or an aldehyde RCHO (Scheme II) are the products of the degradation. Scheme I consists of one hydrolytic mechanism and one mechanism requiring involvement of an oxygenase. Scheme II depicts two oxygenase mechanisms and one hydrolytic process. The degradation product of 2-phenylethanesulfonic acid is 2-phenylethanol when intact *E. coli* cells are used. Dideuterated organosulfonic acid was previously exploited to narrow down the number of possible mechanisms. For instance, 2-phenyl-1,1-dideuteroethanesulfonic acid was converted to 2-phenylethanol which retained only one deuterium atom. This observation removed the mechanisms of Scheme I from further consideration. We now have obtained C-S bond cleavage in cell-free lysate. This step forward now allows us to narrow the mechanistic options to one possibility.

SCHEME I



We discovered that the product ratio of degradation products changed when moving from degradation mediated by intact cells to degradation by cell free lysate. Degradation of 2-phenylethanol by intact *E. coli* cells leads to 2-phenylethanol as the primary product with trace formation of phenylacetic acid. In cell lysate, the dominant product is phenylacetic acid. Only trace amounts of 2-phenylethanol can be detected. The cell lysate in these experiments was prepared from French press lysate of *E. coli* cells grown on 3-phenylpropanesulfonic acid as the sole source of sulfur. These cell-free lysates, after desalting, had an absolute requirement for the addition of NADH for C-S bond cleavage to be observed. This NADH requirement removed the hydrolytic mechanism of Scheme II from further consideration. Of the remaining two mechanisms of Scheme II, one is a monooxygenase process while the other postulates the action of a dioxygenase. Determining whether sulfite or sulfate is the product of C-S bond cleavage would differentiate between dioxygenase and monooxygenase activity.

SCHEME II



An assay was developed where time points were collected and quenched with acid to precipitate protein. After centrifugation, Ellman's reagent was added and color change quantitated spectrophotometrically. In the absence of sulfite, no color formation was observed. This assay clearly established that sulfite is the product of C-S bond cleavage. No reduction of sulfate to sulfite could be detected in control experiments. Carbon to sulfur bond cleavage is thus best formulated as proceeding through the monooxygenase mechanism of Scheme II.

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