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# HYDROXYLATION OF CAMPHOR AND PERICYCLOCAMPHANONE. EVIDENCE AGAINST SUBSTRATE BINDING THROUGH ENOL LINKAGES

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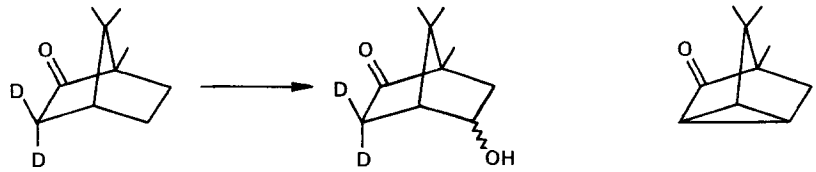
SUMMARY - Hydroxylation of camphor-3,3-$d_2$ in P. Putida occurs without loss of deuterium. Furthermore, pericyclocamphanone, a compound incapable of enolization is also hydroxylated by the same bacterial strain. These results indicate that enolization is not required for substrate binding.

The oxidation of camphor by the P. Putida system has been extensively explored, and details regarding the metabolic sequence are available (1). Briefly, the oxidation of (+) camphor proceeds via the 5-exo-hydroxy-derivative to the 2,5-diketo-compound which then undergoes ring cleavage. The mechanism of binding of camphor to the enzyme is, however, unknown, and the possibility of substrate binding through an enol linkage has been recognized (2,3). Our interest in the enolization of bicyclic ketones (4,5) prompted us to explore this suggestion, and in this communication we present evidence against the involvement of enols in the binding step.

Since enolization is frequently accompanied by isotope exchange, we prepared (+)camphor-3,3-$d_2$ (1) through base catalyzed isotope exchange (5). The isotopic content of the material was 1.96D/molecule as determined by mass spectrometry. The labeled substrate was added to the culture (ATCC #23289) according to procedures outlined earlier (6), and the neutral metabolites were analyzed by GC-chemical ionization mass spectrometry (GC-CI/MS). The

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isotopic content of both 5-exo- and 5-endo-hydroxy-camphor (2) was identical to that of the starting material. Although this result is consistent with an absence of enolization, a similar result could be obtained if the abstracted deuteron was sequestered by the enzyme.

In order to clarify this ambiguity, we metabolized pericyclocamphanone (3), a substrate incapable of enolization. The compound was prepared by converting d-2,3-bornanone to the tosylhydrazone followed by hydrolysis to diazocamphor (7) and subsequent elimination (8), rigorously purified, and metabolized by P. Putida as before. The neutral metabolites in the spent medium were analyzed by GC-CI/MS and shown to contain a number of hydroxy-camphors, one of which appeared to correspond to 5-exo-hydroxy-camphor - the initial product obtained from the metabolism of camphor.

These results, considered together, indicate that substrate binding through an enol linkage is extremely improbable for camphor, and by extension, for other bornanones.

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