A Novel, Site-specific, Aromatic Hydroxylation by Mutants of *Pseudomonas testosteroni*

By GASTON O. DAUMY,* ALEXANDER S. MCCOLL, and GLENN C. ANDREWS
(*Pfizer Central Research, Pfizer Inc., Groton, Connecticut* 06340)

Summary Mutants of *Pseudomonas testosteroni* afford a site-specific aromatic hydroxylation of *m*-hydroxybenzoic acid giving 2,3-dihydroxybenzoic acid.

MANY micro-organisms can use *m*-hydroxybenzoate (MHB) as the sole carbon and energy source for growth. These microbes hydroxylate MHB at either position 4 or 6 prior to ring cleavage.[1] We report a novel biochemical aromatic hydroxylation by mutants of *Pseudomonas testosteroni* ATCC No. 17511. These mutants, isolated for their inability to grow on MHB as the sole carbon source, hydroxylate MHB at position 2 forming 2,3-dihydroxybenzoate (2,3-DHB). This compound was isolated as the crystalline free acid (m.p. 207—208 °C; lit., 207—210 °C)† in 50% yield from a buffered reaction mixture (pH 7·0) containing non-proliferating, washed bacterial cells, 1 g/l of MHB, and lactate, and incubated at 30 °C for 18 h.[2] This report presents the first example of specific biochemical hydroxylation of MHB at position 2. No hydroxylation at position 5 was detected and hydroxylation at positions 4 or 6 would have allowed these mutants to grow on MHB as the sole carbon source since the product of these hydroxylations will serve as growth substrates for both the mutant and the wild type.[2]

Calculations of π-electron distribution in frontier molecular orbitals of MHB, used to predict the site of aromatic hydroxylation by micro-organisms, suggest a greater susceptibility for radical substitution at the 2- compared with the 4- and 6-positions.[3] This has been observed *in vitro* in the hydroxylation of MHB with oxygen in the dihydroxyfumaric acid–peroxidase system whereby, as expected, 2,3-DHB, 2,5-DHB, and 3,4-DHB are all formed.[4] The present observation of the formation of 2,3-DHB is in agreement with the predicted reactivity of MHB to radical substitution *in vitro*. The discovery of this 2-hydroxylase activity, therefore, completes the set of expected *in vivo* hydroxylases.

(*Received*, 31*st July* 1979; *Com.* 833.)

† Physical and spectral characteristics were identical to those of authentic MHB.

[1] B. F. Johnson and R. Y. Stanier, *J. Bacteriol.*, 1971, **107**, 468; Y. N. Karasevich and Y. S. Ivaoylov, *Doklady Acad. Nauk S.S.S.R.*, 1977, **234**, 696; E. E. Groseclose and D. W. Ribbons, *Biochem. Biophys. Res. Comm.*, 1977, **55**, 897; S. Sugiyama, K. Yano, K. Komagata, M. Kazama, and K. Arima, *Bull. Agric. Chem. Soc., Japan*, 1960, **24**, 211; G. Vidal, *Ann. Inst. Pasteur, Paris*, 1969, **117**, 47; M. L. Whellis, N. J. Palleroni, and R. Y. Stanier, *Arch. Mikrobiol.*, 1967, **59**, 302; K. Yano and K. Arima, *J. Gen. Appl. Microbiol.*, 1958, **4**, 241.
[2] Full details of microbiological experimental procedures, isolation, and structure identification will be submitted for publication in the Journal of Bacteriology.
[3] T. Omori and K. Yamada, *Agr. Biol. Chem.*, 1973, **37**, 1809.
[4] D. R. Buhler and H. S. Mason, *Arch. Biochem. Biophys.*, 1961, **92**, 424.