Microbial Hydroxylation of 1,2-(α-Oxotetramethylene)ferrocene

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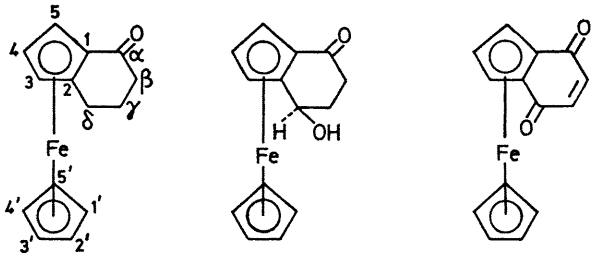
Summary Hydroxylation of α-oxo-1,2-tetramethylene-ferrocene with the mould *Sporotrichum sulfurescens* gave *exo*-6-hydroxyferroceno[1,2]cyclohex-1-en-3-one, demonstrating the capacity for oxygenation of hydrocarbons by enzymic systems in the presence of the ferrocene moiety.

THE variations in substrate structure that can be accommodated by the mould *Sporotrichum sulfurescens* (ATCC 7159) during enzymatic oxygenation reactions are remarkable. The effects of many structural variations on these reactions are as yet unknown. To explore further this aspect of microbial oxygenation reactions, we have prepared the known 1,2-(α-oxotetramethylene)ferrocene[1] (**1**) in order to confront the organism with an organometallic substrate. Our results are outlined below.

Oxygenation of (**1**) with *S. sulfurescens* proceeded most satisfactorily at low substrate-levels. For example, in 10 l fermentations with substrate levels of 0·20 g/l, both starting material and product were obtained, while in



(1) (2) (3)

fermentations at 0·05 g/l, t.l.c. examination showed the substrate to be essentially consumed after 48 h. The product

and unchanged (**1**) were obtained by methylene chloride extraction of the filtered beer. Chromatography of the extract residue on Florisil followed by crystallization of the product from acetone–Skellysolve B gave 0·138 g (from 1·0 g of substrate) of orange product (**2**), m.p. 105—116°. Two additional recrystallizations gave material having m.p. 119—122°. From another fermentation (10 l, 0·20 g/l) there was obtained after the initial crystallization, 0·042 g of product, m.p. 119—123°;† i.r. and n.m.r. spectra identical with those of the above product. The spectral data and the chemical reaction described below allow assignment of structure (**2**) to the fermentation product.

The spectral data clearly show that substrate (**1**) had undergone hydroxylation during the microbial reaction. The position of hydroxylation was shown to be at C_δ when oxidation of (**2**) with activated manganese dioxide[2] gave the known violet ferrocenobenzoquinone (**3**);[3] recrystallization from CH_2Cl_2–Skellysolve B gave crystals, m.p. 150—151·5° (lit. m.p. 146—147°,[3] 146—148°[4]). Formation of a compound intermediate between (**2**) and (**3**) during the oxidation was observed by monitoring the reaction with t.l.c. (20% acetone in chloroform). This orange compound was likely the saturated diketone since its R_F (0·685) was much nearer to that of (**3**) (0·835) than to that of starting keto-alcohol (0·343).

The configuration of the hydroxy-group at C_δ in (**2**) was shown to be *exo* by comparison of the mass spectrum to that reported[4] for the *endo*-isomer of the same compound. In general, a number of differences in the two spectra support the argument that the present compound is isomeric with the reported compound. Specifically, a ratio of 0·1 is found for the intensity of the 138 mass peak to that of the 121 mass peak in the spectrum of (**2**). The magnitude of this ratio has been found to be generally characteristic for the respective isomers[5] and in the present case is consistent with an *exo*-hydroxy-group in (**2**).

The observation of optical activity† for product (**2**), although of relatively low degree by comparison with the rotations obtained for several 1,2-(α-oxotetramethylene) ferrocenes of high optical purity,[6] is, nevertheless, of significance since it permits us to be certain that formation of (**2**) is the result of an asymmetric, and therefore enzymatic, hydroxylation reaction as opposed to an autoxidation of the chemically reactive δ-position. Correlation of the c.d. spectrum of (**2**) with that of optically active (**1**)[6] suggests that a slight excess of the (1*S*)-enantiomer is present.

These experiments demonstrate the ability of *S. sulfurescens* to carry out a hydroxylation reaction at saturated carbon in the presence of an organometallic moiety.

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† C.d. 460 nm (θ −131·5), 385 (0), 338 (−228·0), 327·5 (0), 310 (394·5), 300 (505·0).

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[6] H. Falk and K. Schlögl, *Monatsh.*, 1965, **96**, 1065; for a review of ferrocene stereochemistry, see K. Schlögl, *Topics in Stereochem.*, 1967, **1**, 39.