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# SELECTIVITY AND MECHANISM IN THE MICROSOMAL BENZYLIC HYDROXYLATION

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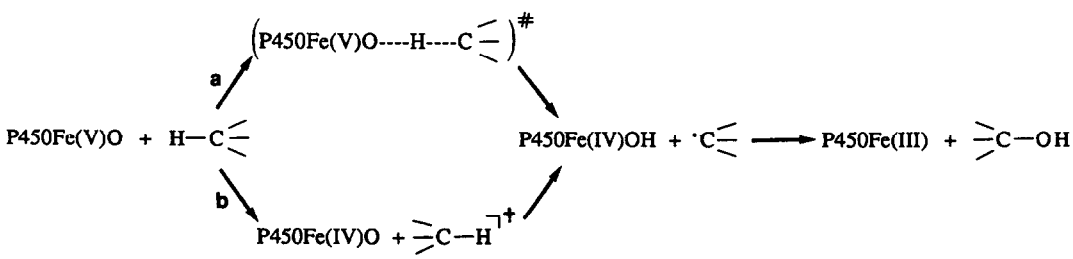
*Summary* : The oxidation by rat liver microsomes of 4-Z-1,2-dimethylbenzenes (**1**) and 4-methoxybenzyltrimethylsilane (**2**) has been investigated. The reaction of the former substrates leads to the expected isomeric benzyl alcohols **3** and **4**, with a very low intramolecular selectivity, and only O-demethylation is observed in the reaction of **2**. These results suggest the operation of a hydrogen atom transfer mechanism.

One of the most important properties of the Cytochrome P450 family of enzymes is the capacity of carrying out the $\geq C-H \longrightarrow \geq C-OH$ conversion with alkanes and alkylaromatic compounds[1]. There is a general agreement on the nature of the active species, an iron(V) oxo-form of the hemoprotein, but the mechanistic aspects of the hydroxylation process are still object of continuous debate particularly with regard to the distinction between the two most likely reaction pathways: that involving a hydrogen atom transfer (HAT) and the one involving an electron transfer (ET), between the oxo complex and the substrate (paths a and b, respectively in the Scheme). In this context particular relevance is attributed to the design and use of mechanistic probes which can provide some insight into the problem[2].

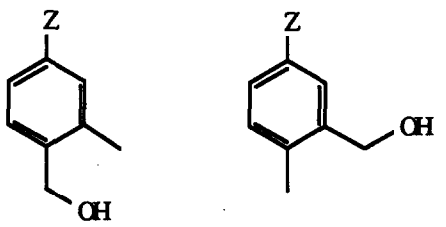



Scheme

We have recently shown that a distinction between HAT and ET mechanisms can be provided, with a reasonable degree of confidence, by studies concerning the intramolecular selectivity of the side chain oxidation of substituted di- and tri-methylbenzenes (generally much higher in ET than in HAT reactions)[3] and the reaction products formed in the oxidation of benzyltrimetylsilanes (desilylation, with formation of benzylic derivatives is observed *only* in ET processes)[4]. Thus, it is seemed of interest to apply these criteria to the problem of the mechanism of the oxidation of alkylaromatics induced by cytochrome P-450 enzymes and accordingly in this note we report on the microsomal oxidation of 4-Z-1,2-dimethylbenzenes (**1**, Z = Cl, t-Bu) and 4-methoxybenzyltrimethylsilane (**2**). These compounds cover a range of oxidation potential values of 0.6-0.8 V[5] and thus should allow a meaningful exploration of the scopes of HAT and ET mechanisms.

Incubation of the substrates (4.5 mM) with phenobarbital induced rat liver microsomes[8] (0.65 g in 6.5 ml of phosphate buffer) and an NADPH generating system (0.65 ml) have been carried out at 35-36 °C for 60 min. The mixture has then been treated with HCl, extracted with chloroform and analyzed by GC and GC-MS (comparison with authentic specimens ).

The microsomal oxidation of **1** leads to the isomeric benzyl alcohols **3** and **4** with a yield of ca 20-30 % with respect to the starting substrate. The **3**/**4** molar ratios are reported




**3** **4**

in the Table where they are compared with the corresponding values obtained in the photochemical chlorination ( a HAT reaction) and in the CAN-promoted side-chain oxidation (an ET process, CAN = cerium(IV) ammonium nitrate) of the same substrates[9].

Table. Intramolecular Selectivity [(3/4) molar ratio] in Some Side Chain Reactions of 4-Z-1,2-Dimethylbenzenes.

| | 3/4 molar ratio | | |
|---|---|---|---|
| Z | oxidation by microsomes[a] | CAN/AcOH[b] | $Cl_2$,hv/$CCl_4$[c] |
| Cl | 1.4 | 13.0 | 2.0 |
| t-Bu | 1.6 | 6.0 | 1.2 |

(a) Average of two or more determinations. For the reaction conditions see text. (b) Ref. 9. In this case **3** and **4** are the corresponding benzyl acetates. (c) Ref. 9. After hydrolysis of the corresponding chlorides.

It can immediately be seen that the microsomal oxidation exhibits a very low intramolecular selectivity. Moreover, the **3/4** molar ratios are quite close to those observed in the photochemical chlorinations and greatly different from those found in the reactions induced by CAN. Clearly, these results suggest a HAT mechanism for the microsomal benzylic oxidation. This conclusion is also supported by the results obtained in the oxidation of **2**. In this reaction no benzylic derivatives form, which excludes an ET mechanism since desilylation to produce a benzyl radical is the expected fate of a benzyltrimethylsilane cation radical[4]. Only the O-demethylation product 4-hydroxybenzyltrimethylsilane is observed (yield ca 5 %), which again is indicative of the operation of a HAT mechanism[10]. Thus, since the $E_p$ of **2** is ca 1.8 V vs NHE, the scope of the HAT mechanism extends to include substrates with an oxidation potential even lower than that attributed ( ca 2 V ) to the oxo-form of cytochrome P-450[11]. It has to be noted that this conclusion is not in line with that of previous investigations which have suggested an ET mechanism for the cytochrome P-450 induced oxidations of heteroatom containing substrates with oxidation potentials up to 2.3 V[10,12].

A HAT mechanism has recently been shown to hold also for the benzylic oxidation promoted by synthetic iron(III) porphyrins and iodosobenzene in $CH_2Cl_2$[13], which confirms the validity of the use of these compounds, in an apolar medium[13b], to mimic the properties of cytochrome P-450. A difference, however, has been noted between the microsomal oxidation and that induced by the model systems: with **2**, O-demethylation is observed in the former case, whereas preferential attack at the benzylic hydrogens takes place when iron(III) porphyrins are used.

It is probable that the lack of reactivity of the benzylic hydrogens in the microsomal oxidation has to be ascribed to an effect of the protein structure[14] which might make difficult an appropriate approach of the bulky $CH_2SiMe_3$ group to the active site of the enzyme.

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