One Electron Oxidation of α-Alkylbenzyl Alcohols Induced by Potassium 12-Tungstocobalt(III)ate − Comparison with the Oxidation Promoted by Microsomal Cytochrome P450

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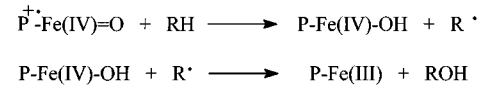
The chemical and microsomal oxidations of a number of 4-methoxy substituted α-alkylbenzyl alcohols 4-MeOPh-CH(R)OH (E_{ox} = 1.6−1.7 V vs SCE) were investigated. Using potassium 12-tungstocobalt(III)ate, a bona fide one electron oxidant, competition between $C_\alpha-H$ and $C_\alpha-C_\beta$ bond cleavage in the intermediate radical cation was observed when the side-chain alkyl group R was Et (**2**) and *i*Pr (**3**). With R = Me (**1**), only C−H bond cleavage took place, whereas with R = *t*Bu (**4**) C−C bond cleavage was the exclusive fragmentation process. In contrast, the microsomal oxidation of the two substrates **3** and **4** led in both cases to the exclusive formation of the corresponding ketone. Thus, an electron transfer mechanism appears unlikely for the microsomal oxidation of α-alkylbenzyl alcohols, even though the oxidation potential of these species is lower than or comparable to that of the active oxidant in the enzyme. A hydrogen atom transfer mechanism is more in line with these results.

Introduction

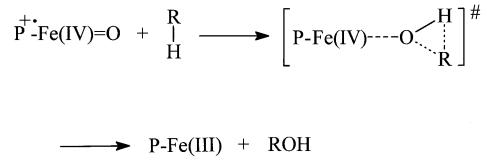
The mechanism of the aliphatic hydroxylation promoted by cytochrome P450 enzymes is attracting continuous interest. With unactivated alkanes as substrates, the hydrogen atom transfer (HAT) mechanism is the one receiving the greatest consensus. According to this mechanism (Scheme 1), the iron oxo complex radical cation, believed to be the active oxidant in the enzyme (abbreviated as $P^{+\bullet}-Fe(IV)=O$, where P is the protoporphyrin IX),[1] abstracts a hydrogen atom from the substrate forming a carbon radical. Fast oxygen rebound between this radical and P−Fe(IV)−OH leads to the hydroxylated alkane.

Scheme 1



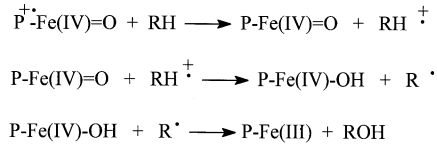
However, doubts about the generality of this mechanism have recently been raised by Newcomb and his associates who presented evidence in favor of a nonsynchronous concerted process (Scheme 2), not involving a real reaction intermediate.[2]

Scheme 2



The matter can become still more complex when dealing with the side-chain hydroxylation of alkylaromatic compounds. The oxidation potential of these compounds is much lower than that of unactivated alkanes and in many instances it can be lower than or comparable to that (1.7−2.0 V vs SCE) estimated for the active oxidant in cytochrome P-450.[3] Thus, in this case, an additional mechanistic possibility can be envisaged, that is the electron transfer mechanism (ET) illustrated in Scheme 3. An alkylaromatic radical cation is first formed which is then deprotonated, presumably by P−Fe(IV)=O, to produce a benzyl radical. The oxygen rebound step follows, as in the HAT mechanism.

Scheme 3



In the last few years, we have addressed the problem of the mechanism of the side-chain hydroxylation of alkylaromatic compounds by cytochrome P-450 and particularly we were involved in the search for evidence indicating the operation of the electron transfer mechanism. Our approach was that of studying the oxidation by microsomal cytochrome P-450 of suitably chosen substrates, such as 4-substituted-1,2-dimethylbenzenes[4], 1,4-dialkylbenzenes[5], benzyltrimethylsilanes[4], which upon electron transfer can give a products pattern different from that expected for a hydro-

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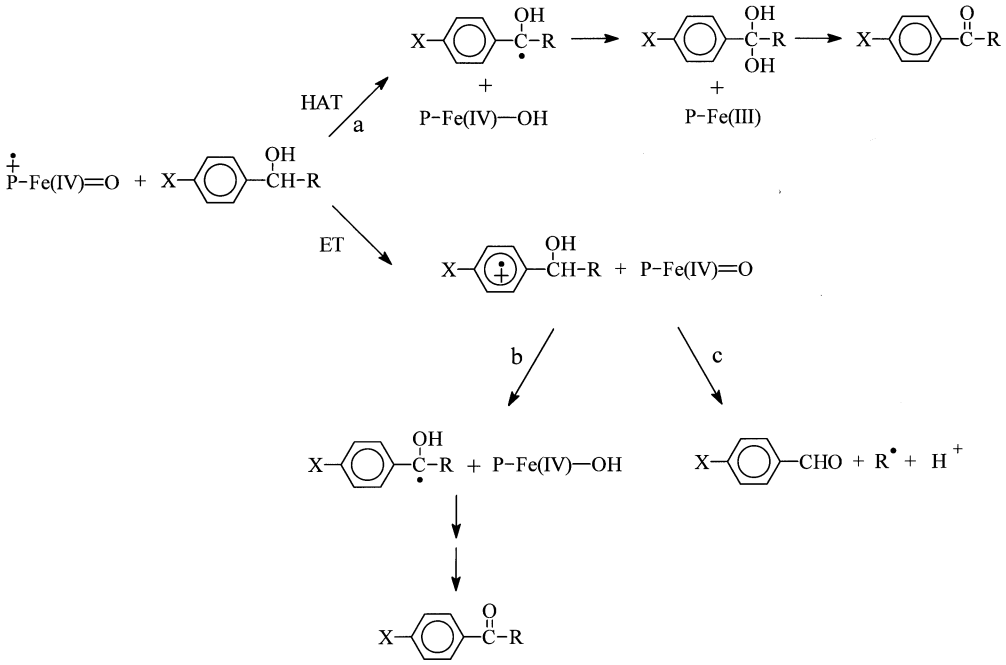
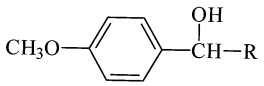
gen atom transfer mechanism.[5][6] These studies did not provide any evidence for the occurrence of an ET mechanism in the oxidation of these substrates, thus leading us to suggest that these reactions probably proceed through the HAT mechanism.

With the aim of getting a further insight on this important problem, we felt, on the basis of the pioneering work by Snook and Hamilton[7] and Trahanowsky and his associates,[8] that the study of the oxidation of α-alkylbenzyl alcohols could provide us with an additional simple and reliable mechanistic criterion to distinguish HAT from ET mechanisms. Accordingly, if the microsomal oxidation takes place by a HAT mechanism, as suggested for aliphatic secondary alcohols[9], an α-hydroxy carbon radical is produced which should undergo fast oxygen rebound to form a gem-diol and then the expected ketone (path a in Scheme 4). However, if an ET mechanism is operating, an aromatic radical cation is formed, which can be deprotonated or undergo C-C bond cleavage. So, as shown in Scheme 4 (paths b and c, respectively) the formation of ketone can be accompanied by that of benzaldehyde. Of course, the ratio between the two products will depend on the nature of the alkyl group R.

Scheme 4



Thus, following this idea, we have first investigated the oxidation of the α-alkylbenzyl alcohols **1**−**4** by a genuine one electron transfer oxidant, viz. the potassium 12-tungstocobalt(III)ate, $K_5Co^{III}W_{12}O_{40}$, from now on indicated as $Co^{III}W$,[10][11][12] to get information on the behaviors of these substrates in these reactions. On the basis of this information, the oxidation of the alcohols **3** and **4** by microsomal cytochrome P-450 has then been carried out. Alcohols **1**−**4** have an oxidation potential around 1.6-1.7V,[13] which makes thermodynamically favorable an electron transfer mechanism for their reaction with cytochrome P-450. The results of this investigation are reported in the present paper.



1 R = CH_3
2 R = CH_2CH_3
3 R = $CH(CH_3)_2$
4 R = $C(CH_3)_3$

Results and Discussion

ET Oxidations Induced by $Co^{III}W$: The reactions of substrates **1**−**4** with $Co^{III}W$ were performed at 50°C under an argon atmosphere in $AcOH/H_2O$ (55:45) (w/w) in the presence of AcOK, using a 1:1 substrate/oxidant molar ratio. The oxidation of **1** gave 4-methoxyacetophenone as the exclusive product, that of **2**−**3** afforded both the corresponding ketone and 4-methoxybenzaldehyde. The latter was the exclusive product in the oxidation of **4**. The absolute yields (referred to the oxidant) and the product distributions are reported in Table 1.

Since $Co^{III}W$ is a genuine electron transfer oxidant, the data in Table 1 can be interpreted on the basis of the formation of the intermediate radical cation 4-MeOPhCH(OH)$R^{+\bullet}$. When R = Me, the radical cation exclusively undergoes deprotonation (Scheme 5, path b). The α-hydroxy carbon radical is then oxidized by $Co^{III}W$ to give 4-methoxyacetophenone as the only observed product. When R = Et, deprotonation is no longer the exclusive reaction path of the radical cation, but C−C bond cleavage,

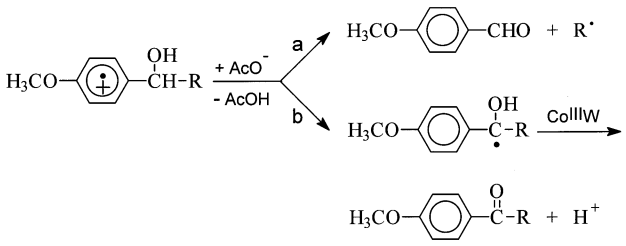
Table 1. Product distribution and total yield for the oxidation of alcohols **1**−**4** [4-MeOPhCH(R)OH] (0.05 M) with Co(III)W (0.05 M) in AcOH/H_2O (55:45, w/w) at 50°C in the presence of AcOK (0.30 M) [a]

Compound	R	4-MeOPhCHO [%]	4-MeOPhCOR [%]	Yield [%][b]
1	CH_3	−	> 99	60
2	CH_2CH_3	15	95	67
3	$CH(CH_3)_2$	39	61	72
4	$C(CH_3)_3$	> 99	−[c]	90

[a] The average error is 10% of the reported value. − [b] Referred to the oxidant. − [c] Detected in traces.

assisted by the α-OH group[15] (Scheme 5, path a) begins to compete and substantial formation of 4-methoxybenzaldehyde is observed. The relative importance of this C−C bond cleavage path, which is expressed by the aldehyde to ketone ratio, increases as we move to R = *i*Pr and then to R = *t*Bu. In the latter case, 4-methoxybenzaldehyde is the only product, indicating that the radical cation undergoes C−C bond cleavage exclusively.

Scheme 5



Certainly, in the radical cation, the importance of the C−C bond cleavage path should depend on the stability of the alkyl radical formed in this process. Thus, the aldehyde/ketone ratio increases in the order Me < Et < *i*Pr < *t*Bu, which is the order of increasing stability of the alkyl radicals formed by C−C bond fragmentation.[16]

However, the difference in the aldehyde/ketone ratio between R = *i*Pr and R = *t*Bu seems too large (0.64 in the former case and > 99 in the latter, a more than 100 times increase!) to be accounted for only on the basis of the different stability of isopropyl and and *tert*-butyl radicals. Accordingly, the aldehyde/ketone ratio increases of only 10 times on going from **2** (R = Et) to **3** (R = *i*Pr), in spite of the fact that, at least judging from the bond dissociation energies of the pertinent C−H bonds in the parent hydrocarbons,[17] the difference in stability between ethyl and isopropyl radicals is larger than that between isopropyl and *tert*-butyl radicals. Therefore, some additional factor should play a role in determining the competition between the two reaction pathways of the radical cation.

We suggest that this additional factor is represented by the bulkiness of the R group, which can exert a twofold action: first, it can favour the C−C bond cleavage pathway indirectly by depressing the rate of the competitive deprotonation process. Accordingly, a bulky R group is expected to increase the energy of the conformation (C−H bond collinear with the aromatic π system) which provides the best overlapping between the π system and the scissile bond (stereoelectronic effect). Convincing experimental evidence in this respect is available.[19] Second, the steric interactions present in the radical cation ground state can be much better relieved by C−C than by C−H bond cleavage. This effect too should favour C−C bond cleavage more when R = *t*Bu than when R = *i*Pr, in view of the higher steric requirements of the former group ($E_s = -1.57$) than of the second group ($E_s = -0.47$).[20]

Reactions with Microsomal Cytochrome P-450: The extensive C−C bond cleavage observed for the one-electron oxidation of **3** and **4** by $Co^{III}W$, makes both these alcohols and particularly the latter very suitable mechanistic probes to detect the occurrence of ET mechanisms. Therefore **3** and **4** were subjected to oxidation by microsomal cytochrome P-450 using phenobarbital induced rat liver microsomal preparations in the presence of a NADPH generating system ($NADP^+$ glucose 6-phosphate/glucose 6-phosphate dehydrogenase). In both cases the only product observed was the corresponding ketone, 1-(4-methoxyphenyl)-2-methyl-1-propanone from **3** and 1-(4-methoxyphenyl)-2,2-dimethyl-1-propanone from **4** (Table 2). With both substrates no evidence for the formation of fragmentation products was obtained. Particularly striking is the comparison between the microsomal and the $Co^{III}W$ induced oxidations of **4**: exclusive C−H bond cleavage in the former case, exclusive C−C bond cleavage in the second.

Table 2. Product yield of the microsomal oxidation of alcohols **3** and **4** carried out at 36°C in a phosphate buffer pH = 7.4 in the presence of a NADPH generating system[a]

Compound	R	4-MeOPhCOR Yield [%][b]
3	$CH(CH_3)_2$	27
4	$C(CH_3)_3$	17

[a] $NADP^+$ glucose 6-phosphate/glucose 6-phosphate dehydrogenase. − [b] Referred to the substrate.

Thus, the microsomal oxidation of **3** and **4** produces a product distribution completely different from those observed when the same substrates were reacted with $Co^{III}W$. These results clearly speak against the operation of an ET mechanism in the microsomal oxidation of **3** and **4**, in spite of the fact that, as mentioned above, such a mechanism should be thermodynamically feasible.

Of course, at least in principle, the possibility should be considered that the enzymatic environment is much more basic than the medium (AcO^-/AcOH) used in the $Co^{III}W$ induced reactions. Thus, were a radical cation formed in the enzymatic environment it might undergo C-H bond cleavage at a much faster rate than C−C bond cleavage, thus explaining the above results. This possibility, however, appears highly unlikely, especially in the case of $\mathbf{4}^{+\cdot}$, since pulse radiolysis experiments have recently shown that deprotonation of this radical cation is an extremely sluggish process even in basic media, while a rate constant as high

as 1.8×10^5 s^{-1} for the C−C bond cleavage pathway has been determined.[21]

Thus, we can conclude with reasonable confidence that the microsomal oxidation of α-alkylbenzyl alcohols of oxidation potential ≥ 1.7 V does not occur by an ET mechanism, but most probably by a HAT mechanism.[22] Since this conclusion can be extended as well to the side-chain oxidation of alkylaromatics with comparable oxidation potentials, the present study fully supports our previous conclusions based on different mechanistic probes.

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Experimental Section

GLC analyses were performed on a Varian 3400 GLC using a 25m × 0.2mm silica capillary column coated with methylsilicone gum. GLC-MS analyses were performed on a HP5890 GLC equipped with a 12m × 0.2mm silica capillary column coated with methylsilicone gum and coupled with a HP5970 MSD. ^{1}H-NMR spectra were recorded on a Bruker WP80 SY and on a Bruker AC 300 P spectrometer.

Materials: Potassium 12-tungstocobalt(III)ate was prepared as described previously.[11] 4-Methoxybenzaldehyde, 4-methoxyacetophenone and 4-methoxypropiophenone were used as received.

Substrates were generally identified by GLC-MS and ^{1}H NMR. The following substrates were prepared according to previously described procedures: 1-(4-methoxyphenyl)-1-ethanol (**1**),[7] 1-(4-methoxyphenyl)-2,2-dimethyl-1-propanol (**4**).[15] 1-(4-Methoxyphenyl)-1-propanol (**2**) was prepared by reaction of the corresponding ketone with $NaBH_4$ in isopropyl alcohol; 1-(4-methoxyphenyl)-2-methyl-1-propanol (**3**) by reaction of isopropylmagnesium bromide with 4-methoxybenzaldehyde.[23] 1-(4-Methoxyphenyl)-2-methyl-1-propanone and 1-(4-methoxyphenyl)-2,2-dimethyl-1-propanone were prepared by reaction of 4-methoxy-phenylmagnesium bromide with the appropriate acid chloride (isobutyryl chloride and trimethylacetyl chloride, respectively).[15]

Microsomal Preparation: The liver microsomes were obtained from male Sprague-Dawley rats pre treated with sodium phenobarbital (300 mg kg^{-1} of body weight, each day for 7 days) according to a procedure reported in the literature.[24]

Products Analysis: Products were generally identified by GLC (comparison with authentic specimens) and by GLC-MS analysis.

Oxidation with $Co^{III}W$: The reactions were performed at 50°C under Argon atmosphere in AcOH/H_2O (55:45) (w/w) using the following conditions: substrate (0.05 M), $Co^{III}W$ (0.05 M), AcOK (0.30 M). Workup was performed as described previously[10] and the residue was subjected to GLC analysis. The stability of the substrates to reaction conditions was shown by blind reactions in AcOH/H_2O (55:45) at 50°C. Product distribution and yield referred to substrates **1**−**4** are reported in Table 1.

Enzymatic Reactions: In the microsomal oxidation, phenobarbital induced rat liver microsomes (35 mg of protein), NADPH generating system (5 mg of $NADP^+$, 25 mg of glucose 6-phosphate, 0.2 mg of a 1mg/ml solution of glucose 6-phosphate dehydrogenase in 3.2 M ammonium sulfate buffer) and substrate (9 μmol) were incubated in 5 ml of 0.1 M phosphate buffer (pH = 7.4) at 36°C for 5 minutes. Reaction products were extracted as reported previously[4] and analysed by GLC and GLC-MS. The only product observed in the oxidation of **3** and **4** was the corresponding ketone (2.4×10^{-6} mol and 1.5×10^{-6} mol respectively). Blank experiments, carried out in the absence of microsomes or of NADPH generating system lead to the complete recovery of the starting material.

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[22] Of course, also the nonsynchronous concerted mechanism proposed by Newcomb and his associates[2] for the oxidation of unactivated alkanes would be consistent with our results. However, with alkylbenzenes and α-alkylbenzyl alcohols, the relatively high stability of benzyl radicals should probably favor the formation of these species as reaction intermediates, so promoting the HAT mechanism.
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