190. Chemistry of Superoxide Ion as Revealed by the Differential Oxidation of Arylpyruvates

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Dedicated to *A. Dreiding* on the occasion of his 60th birthday

(3.XI.78)

Summary

Superoxide ion apparently reacts with acidic substrates *via* species such as O_2, $HO_2^{\cdot}$, O_2^{-}, HO_2^{-} and H_2O_2. Arylpyruvates give arylacetates and arylaldehydes indicating competing nucleophilic and free radical oxidation. Benzaldehyde is further oxidized by free radical and nucleophilic dioxygen species giving benzoic acid. *p*-Hydroxybenzaldehyde gives the corresponding benzoic acid which is best accounted for by $HO_2^{\cdot}$, since O_2^{-} and O_2 are without effect. Hydroquinone is also produced presumably by nucleophilic attack of HO_2^{-}. Replacement of the acidic hydrogen atoms by sodium changes the product distribution in accord with these findings.

One-electron reduction of O_2 is a possible way in which dioxygenases might activate oxygen for reaction with organic substrates [1]. The resulting superoxide radical anion O_2^{-} can be regarded as a bifunctional reagent since it may behave as a free radical or a nucleophile [2]. As it can donate or accept an electron, it should behave both as a reducing and an oxidizing agent. Although examples of its reducing [3], basic [4] and nucleophilic [5] properties have been cited, little is known of its chemical reactivity [2]. In particular, its oxidizing capability is questionable. Apparently, organic substrates are only oxidized if a stable free radical is formed [6]. We now show that oxidation by O_2^{-} is complex, especially when proton donors are present. Apart from O_2^{-}, dismutation yields O_2, H_2O_2, hydroperoxide anion (HO_2^{-}) and significantly, hydroperoxy radical ($HO_2^{\cdot}$).

With arylpyruvic acid (**1**), differentially oxidized products (**2–5**) are obtained which characterize the reactive dioxygen intermediates *(Table)*.

The reaction of phenylpyruvic acid (**1a**) and its monosodium salt with potassium superoxide in dimethylsulfoxide (DMSO) gives phenylacetic and benzoic acids (**2a**

Scheme 1

$$\cdot O{-}O^{-} + H^{+} \rightarrow \cdot O{-}OH$$

$$\cdot O{-}O^{-} + \cdot O{-}OH \rightarrow O_2 + {}^{-}O{-}OH$$

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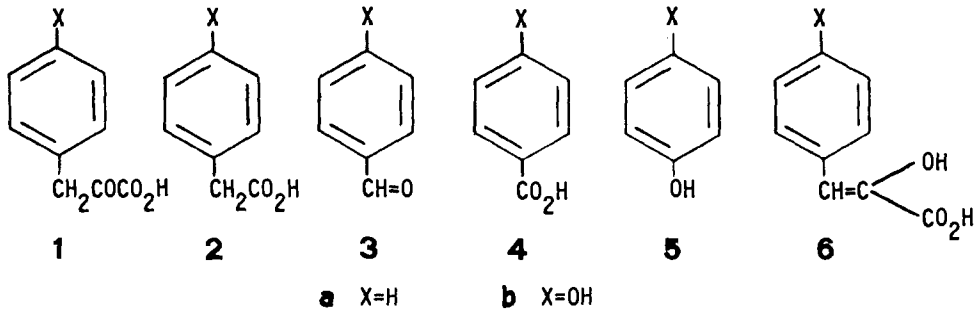


and **4a**) which represent respectively initial oxidative decarboxylation of the α-keto acid and complete oxidation of the side chain *(entries 1* and *2)*. In fact, submission of **2a** to the reaction conditions affords benzoic acid (**4a**) *(entry 9)*, presumably *via* benzaldehyde (**3a**) *(entry 10)*[1]. This result is confirmed by the behaviour of *p*-hydroxyphenylpyruvic acid (**1b**) which gives the *p*-hydroxy derivatives of phenylacetic acid, benzaldehyde, benzoic acid and phenol (**2b**–**5b**) *(entry 3)*[2]).

Table. *Reactions of potassium superoxide*[a]) *with phenylpyruvic acid* (**1a**), *phenylacetic acid* (**2a**) *and benzaldehyde* (**3a**) *and their* p*-hydroxy derivatives* (**1b**, etc.)

Entry	Reactant	Phenylacetic[b]) acid (**2a** or **2b**)	Benzaldehyde[b]) (**3a** or **3b**)	Benzoic[b]) acid (**4a** or **4b**)	Phenol[b]) (**5a** or **5b**)
1	**1a**	3	–	97	–
2	**1a** (Monosodium salt)	8.5	–	91.5	–
3	**1b**	15.5	26	42	15.5
4	**1b** (Monosodium salt)	20.5	46	16	17.5
5	**1b** (Disodium salt)	24	59.5	7.5	8.5
6	**1b** (Trisodium salt)	31	64	2	3
7	**1b** + *t*-Butyl alcohol	10.5	3.5	9	77
8	**2b**	100	–	–	–
9	**2a**	70	–	30	–
10	**3a**	–	–	100	–
11	**3b**	–	85	10	5
12	**3b** (Sodium salt)	–	100	–	–

[a]) Reactions were performed in degassed DMSO under a static atmosphere of N_2. After 24 h reaction with 8 equiv. of KO_2 and 0.25 equiv. of 18-crown-6 ether, the reaction mixture was quenched by addition to aqueous ascorbic acid. Products were rapidly extracted with ethyl acetate. Their identity was established initially by isolation and by TLC. in later runs.
DMSO would reduce long-lived peroxy intermediates [21]. Reduction products were not detected.

[b]) Yields (average of 2 runs) were estimated from ^{1}H-NMR. spectra of crude products.

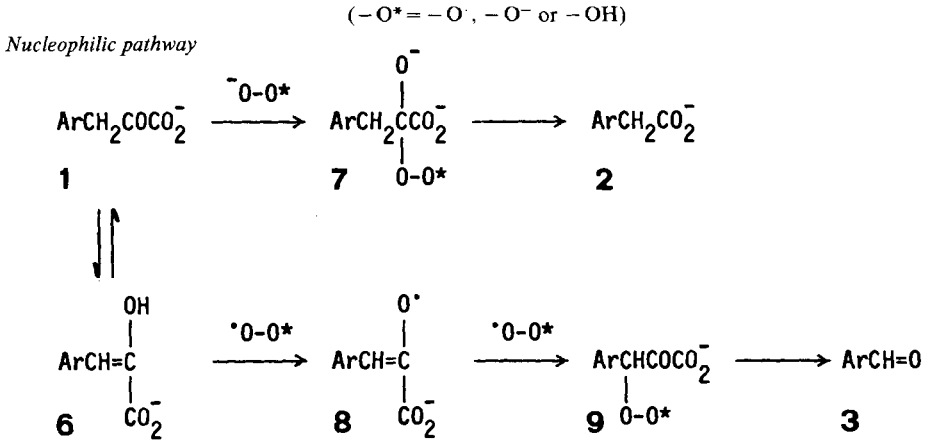
[c]) Reaction performed as in *entry 3*, but with 8 equiv. of *t*-butyl alcohol added.

[1]) Although not essential to the ensuing discussion, the oxidation of **2a** to **4a** probably occurs as shown:

$$ArCH_2CO_2^- \xrightarrow{\text{an oxy radical}} ArCHCO_2^- \rightarrow Ar{-}\underset{\displaystyle O{-}O^-}{\underset{|}{C}}HCO_2^- \rightarrow ArCH{=}O \xrightarrow{\text{any oxidizing species}} ArCO_2H$$

[2]) The absence of homogentisic acid means that this reaction is not a model for the enzymic process [7].

Scheme 2



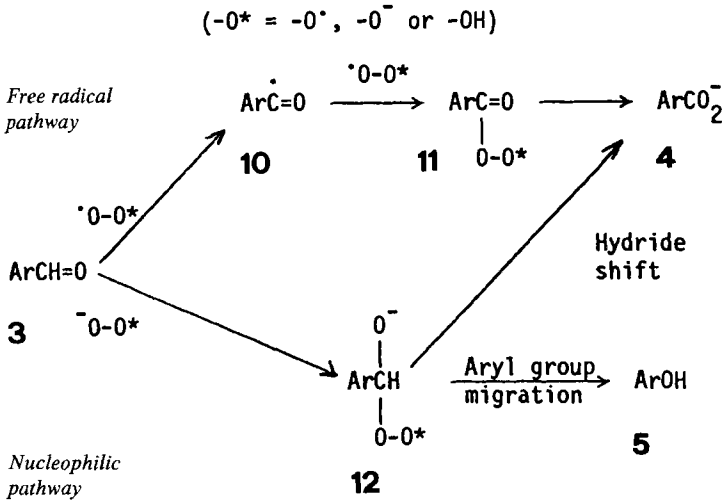
The inertness of the acid **2b** *(Table, entry 8)*[3]) and the fact that aldehyde **3b** is further oxidized to **4b** and **5b** under the reaction conditions point to two distinct oxidation pathways which originate at the α-keto and benzylic carbon atoms respectively. Such a dichotomy is consistent with the bifunctional nature of $O_2^{\cdot-}$. Nucleophilic attack by $O_2^{\cdot-}$ on the α-keto group of **1b** will be responsible for oxidative decarboxylation to **2b** [8] [9]. However, if $O_2^{\cdot-}$ behaves like a free radical, it could be argued that arylaldehyde would be oxidized in similar fashion to the oxygen-mediated process [10].

Rationalization of these results solely in terms of $O_2^{\cdot-}$ would, however, constitute an oversimplification. Thanks to its tautomerization to the enolic acid **6b**, each molecule of *p*-hydroxyphenylpyruvic acid (**1b**) can surrender any one of 3 protons to $O_2^{\cdot-}$. Therefore it is not possible to distinguish between mechanisms involving $O_2^{\cdot-}$ alone and those arising from dismutation products [11] (see *Scheme 1*). We propose that oxidative decarboxylation of arylpyruvate (**1**) may be initiated by nucleophilic attack of either $O_2^{\cdot-}$ or HO_2^- *(Scheme 2)* [12]. The resulting tetrahedral intermediate **7** (−O* = −OH) will lose HO^- and CO_2 giving arylacetate **2** [13]. The corresponding peroxy tetrahedral intermediate **7** (−O* = −O·) resulting attack by $O_2^{\cdot-}$ will eventually lead to the same products as more $O_2^{\cdot-}$ reduces the radical **7** to its anion [20]. Protonation and fragmentation will occur on work-up.

Benzylic oxidation will occur on **1** or the enol **6**. Hydrogen atom abstraction by dioxygen radicals affords the enol radical **8**. Subsequently, **8** on combination with any dioxygen radical gives the α-peroxycarbonyl species **9**. When **9** is a peroxy radical (−O* = −O·), further reduction will give the peroxide anion (−O* = −O⁻) which on protonation affords the α-hydroperoxycarbonyl species **9** (−O* = −OH). Fragmentation of the latter affords HO^-, the oxides of carbon

[3]) The *p*-hydroxy substituent or rather its corresponding anion renders the arylacetate resistant to benzylic oxidation (*cf. Table, entries 8* and *9*); for a discussion on why this is so see [16] and [18].

Scheme 3



[10] [11] and benzaldehyde (**3**) which is further oxidized to benzoic acid (**4**) and phenol (**5**) in amounts characteristic of the dioxygen species present.

This scheme also nicely accounts for the shift in product composition observed when the acidic hydrogen atoms of *p*-hydroxyphenylpyruvic acid (**1b**) are progressively replaced by sodium[4]) *(Table, entries 3–6)*. The formation of increasing amounts of *p*-hydroxyphenylacetic acid (**2b**) at the expense of the more oxidized products (**3b**–**5b**) can be ascribed to the greater availability of $O_2^{\cdot-}$ as dismutation diminishes. Although $O_2^{\cdot-}$ easily causes oxidative decarboxylation (**1b**→**2b**), it must be less capable than triplet O_2 or $HO_2^{\cdot}$ of oxidizing the enol **6** to the enoxy radical **8**. $O_2^{\cdot-}$ may be completely incapable of oxidizing **6** to **8**. The persistence of benzylic oxidation even in the absence of protons could be due to O_2 arising *in situ* by reduction of the peroxy radical **7** or **9** (where $-O^* = -O\cdot$) by $O_2^{\cdot-}$.

The accumulation of arylaldehyde (**3b**) to the detriment of its oxidation products **4b** and **5b** must also spring from less dismutation. Indeed, the fate of arylaldehyde **3** reveals the nature of the dioxygen species and their concentration dependence on proton levels *(Scheme 3)*. We again propose that the products arise by two pathways involving dioxygen species behaving as radical and nucleophilic reagents *(Scheme 3)*. Our argument is based on the fact that HO_2^- [15] and O_2 [16] oxidize benzaldehyde by different mechanisms. Consequently, dioxygen radicals will combine with the benzoyl radical **10** to form the peroxy species **11** which gives just benzoic acid **4**. Clearly, abstraction of a hydrogen atom to create **10** requires a powerful radical initiator *(v. infra)*. On the other hand, dioxygen nucleophiles like HO_2^- will generate the tetrahedral intermediate **12**, which decomposes

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[4]) One, 2 or 3 equivalents of 1N NaOH are added to *p*-hydroxyphenylpyruvic acid. Immediate freezing and lyophilization affords the mono, di and trisodium salts. The last salt shows no hydroxyl absorption in its IR. spectrum, confirming the complete removal of water.

to benzoic acid **4** or the phenol **5**, depending on the nature of the aryl group [15]. The previous observation that benzaldehyde (**3a**) and its *p*-hydroxy derivative (**3b**) on treatment with alkaline H_2O_2 give exclusively benzoic acid (**4a**) and hydroquinone (**5b**) respectively [15] [16] is the consequence of the superior migratory aptitude of the *p*-hydroxyphenyl group. Here, this product difference permits a distinction between free radical and nucleophilic oxidation of **3b**.

Reactions performed on the sodium salt of *p*-hydroxybenzaldehyde (**3b**) *(entry 12)* and benzaldehyde (**3a**) *(entry 10)* show that the former is inert, whereas the latter gives benzoic acid (**4a**)[5]. If an excess is not used, reduction of the tetrahedral intermediate **12** ($-O^*=-O\cdot$) to the anion ($-O^*=-O^-$) may not be achieved [19]. We therefore conclude that $O_2^{\cdot-}$ oxidizes arylaldehydes by the nucleophilic pathway *(Scheme 3)*. When a *p*-hydroxy substituent is present as in **3b**, the tetrahedral intermediate **12** cannot form for electronic reasons. The inertness of **3b** towards $O_2^{\cdot-}$, in the absence of proton donors, is also noteworthy where it appears as a product in the reaction of the trisodium salt of **1b** *(entry 6)*. In contrast, when protons are available from the hydroxyl and carboxyl groups of **1b** *(entry 3)* or the hydroxyl group of **3b** *(entry 11)* then both *p*-hydroxybenzoic acid (**4b**) and hydroquinone (**5b**) are formed. When proton levels are lowered *(entries 4–6)*, the amounts of these 2 products decrease accordingly.

The formation of hydroquinone (**5b**) is easy to understand: HO_2^-, produced by dismutation, nucleophilically attacks **3b** giving **12b** ($-O^*=-OH$) which subsequently rearranges to **5b** *via* the *Dakin* reaction [15] *(Scheme 3)*. The formation of *p*-hydrobenzoic acid (**4b**) under these conditions is difficult to explain at first sight as $O_2^{\cdot-}$ is incapable of oxidizing the aldehyde **3b**. Moreover, O_2 itself is also without effect on **3b** (bubbling O_2 for 24 h through an alkaline solution of **3b** in DMSO). Clearly, some other free radical oxidant is at work. $HO_2^{\cdot}$ is the best candidate [17], since it is generated under the reaction conditions and its concentration should vary with proton levels. Other peroxy derivatives, *e.g.* **9**, **11** or **12** (where $-O^*=-O\cdot$), could also be envisaged, but their participation would be at odds with the correlation of product composition and proton levels.

$HO_2^{\cdot}$ is a short-lived species whose reaction is probably confined to the solvent cage [10]. Nevertheless, it should, by analogy with other neutral peroxy radicals, not only initiate and propagate autoxidation of the aldehyde [16], but also behave as a weak electrophile [18] and should react readily with an electron-rich aldehyde such as **3b**.

A revealing test is the use of an external source of protons for promoting dismutation. The addition of *t*-butyl alcohol [19] in large excess to the reaction of **1b** *(entry 7)* makes hydroquinone (**5b**) the main product. The fact that more of the arylacetic acid (**2b**) is not formed despite the high concentration of HOO^- is due most likely to the rapidity of the benzylic oxidation of enol **6** by the other product of dismutation, O_2 *(Scheme 2)*.

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[5] A recent claim [4] that benzaldehyde is inert to $O_2^{\cdot-}$ in dry pyridine are in contrast to our findings with DMSO as solvent.

Our results show that O_2^- in non-aqueous solvents is a poor oxidant, except where it can act as a nucleophile. When protons are available, O_2^- becomes a good oxidizing system by generating HOO· which dismutates into O_2 and H_2O_2.

We are indebted to the *Swiss National Science Foundation* for support of this research (grant No 2.430.0.75).

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