The Chemical Reactions of Superoxide with Halopyrimidines

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Halopyrimidines such as 2-chloro-, 5-bromo-, and 4,6-dichloro-5-nitropyrimidine undergo substitution reactions with superoxide anion radical (superoxide) to give the corresponding hydroxypyrimidines under suitable conditions. Parallel experiments employing hydroxide instead of superoxide strongly indicate that the reactivity of superoxide is comparable to that of the hydroxide in the reaction with halopyrimidines. The results seem to provide a piece of information in favor of the nucleophilic substitution rather than electron-transfer mechanism in the title reaction.

Introduction

Superoxide, $O_2^{\cdot-}$, is capable of reacting with a variety of substrates owing to its anionic, radical, and redox nature although the nucleophilic and reducing (electron-transfer) process appear to be the predominant reaction. The chemistry of superoxide has been well documentd.[1-3] Chemical studies have employed either electrogenerated superoxide or a suspension of KO_2, which is commercially available. In the case of KO_2, aprotic solvents are used, since superoxide is quite sensitive to protons. Recently there has been growing interest in the reactivity of superoxide with organic compounds, originally intended to elucidate the interactions between oxygen metabolites and complex biochemical systems[4,5] It needs to be noted however that reactions of superoxide with organic compounds are still in the initial stage, in view of the increasing demand of the possible roles of superoxide *in vivo*.[6-8] The purpose of this work is designed to study possible reactions between superoxide and biologically active halopyrimidines. Furthermore, the reaction of superoxide with halopyrimidnes can be used to study the reaction pathways also. In the reaction of superoxide and organic substrates which have leaving groups, two mechanisms have been proposed. One is the aromatic nucleophilic substitution (equation 1-3).[9,10]

$$ArX + O_2^{\cdot-} \rightarrow ArOO\cdot + X^- \quad (1)$$

$$ArOO\cdot + O_2^{\cdot-} \rightarrow ArOO^- + O_2 \quad (2)$$

$$ArOO^- \rightarrow \rightarrow ArOH \quad (3)$$

The other is the electron transfer mechanism (equation 4-8).[11,12]

$$ArX + O_2^{\cdot-} \rightarrow [ArX]^{\cdot-} + O_2 \quad (4)$$

$$[ArX]^{\cdot-} \rightarrow Ar\cdot + X^- \quad (5)$$

$$Ar\cdot + O_2 \rightarrow ArOO\cdot \quad (6)$$

$$ArOO\cdot + O_2^{\cdot-} \rightarrow ArOO^- + O_2 \quad (7)$$

$$ArOO^- \rightarrow \rightarrow ArOH \quad (8)$$

However the mechanism in the reaction of superoxide with activated substrates has been controversial. In the case of halonitrobenzenes, Saveant and co-workers[9,10] propose aromatic nucleophilic substitution(S_NAr) mechanism using electrochemical techniques. While other workers[11,12] propose electron transfer mechanism from the experiments using ^{18}O-enriched KO_2. The work we report hereafter is an attempt to seek an additional piece of information regarding the reaction mechanism in the reaction of superoxide with halopyrimidines, whose reactivity is supposed to be comparable to that of halonitrobenzenes.[13] For the purpose reactivities of KO_2 and KOH towards halopyrimidines will be utilized.

Experimental

Materials and Apparatus. All the chemicals were purchased and used without further purification unless otherwise specified. Melting points were taken on an Electrothermal Melting Point Apparatus. Acetonitrile and DMSO were refluxed over calcium hydride for 3 hrs., and distilled just before use. Thin layer chromatography was performed with Merck silica gel (Art. 7739) and column chromatography was performed using Merck silica gel (Art. 7733). Infrared spectrum were recorded on a Jasco A-1 model. ^1H NMR spectra were taken on a Varian EM 360-A spectrophotometer using tetramethylsilane as an internal standard. UV/VS spectra were measured on a Beckmann UV 5260 model.

Reaction of 1 with KO_2.

(a) At 15°C. In an AtmosBag filled with nitrogen was added **1** (0.114g, 1 mmol) to the mixture of KO_2 (0.142 g, 2 mmol) and dicyclohexano-18-crown 6 (0.744 g, 2 mmol) in acetonitrile (10 m*l*). After 3 days of reaction, UV/VIS, GC, and tlc showed most of the materials to be starting compound.

(b) At 60°C. The procedure followed was the same as described at room temperature except the amount of the materials. In this experiment 1.140 g (10 mmol) of **1** and 1.420 g (20 mmol) of KO_2 were used. Work-up procedure is as follows: After 3 days, when the starting material had disappeared, the mixture was made to pH = 2-3 by adding 5% HCl solution. The aqueous layer obtained upon evaporation of acetonitrile was extracted with ether. Evaporation of ether gave the residue which was next subjected to the column chromatography (2.5 × 15 cm). For the purpose silica gel and chloroform were used. Since the product stayed on top, the silica gel adsorbing product was extracted with ethanol, followed by evaporation gave the product (0.308 g, 32% mp 179-181 °C, Ref. 14 mp 179-180 °C, λ_{max} = 298 nm (H_2O)).

Reaction of 6 with KO_2

(a) At 15°C. The procedure is the same as described in the reaction of **1** with KO_2 at room temeprature except the amount of materials. In this experiemnt 0.970 g (5 mmol) of **6** and 1.065 g (15 mmol) of KO_2 were used. After 2 days of reaction, tlc showed that the conversion was almost com-

Table 1. Reaction of 1 and 2 with KO_2/KOH

Substrate[a]	Nucleophile	Temp (°C)	Time (day)	Procuct[b]	Yield (%)
1	KO_2	15	3	**3**	—
2	"	"	"	—	—
1	KOH	"	"	**3**	—
2	"	"	"	—	—
1	KO_2	60	"	**3**	32[c]
"	KOH	"	"	"	30[c]
"[d]	"	90	2	"	100[e]
2[d]	"	"	"	—	"

[a] **1** = 2-Chloropyrimidine. **2** = 5-Bromopyrimidine. [b] **3** = 2-Hydroxypyrimidine – = Not identified. [c] Isolated yield. [d] in aqueous solution. [e] Checked by tlc.

Table 2. Reactions of 6 with KO_2/KOH

Substrate[a]	Nucleophile[b]	Temp (°C)	Time (hour)	Product Yield (%)[c]	
				7	**8**
6	KO_2	15	48	80	Trace
"	KOH	"	"	"	"
"	KO_2	60	10	90	None
"	KOH	"	"	"	"

[a] **6** = 4,6-Dichloro-5-nitropyrimidine. [b] 3 Equivalent. [c] Isolated Yield **7** = 4,6-Dihydroxy-5-nitropyrimidine **8** = 4-Chloro-6-hydroxy-5-nitropyrimidine.

plete. The insoluble material from which **7** (0.621 g, 79%) was obtained was filtered off. Evaporation of acetonitrile gave a mixture consisting of mostly **7** and **8** in trace. **7**: mp 300 °C (Ref.[15] mp > 300 °C); λ_{max} = 324 nm (H_2O); IR (KBr) 3400 (*m*), 3200-2500 (s), 1750-1620 (*s*), 1550 (*s*), 1350 (*s*), 1280 (*s*), 880(*m*) cm^{-1}; ^1H NMR(DMSO-d_6) δ 8.8(*s*, 1H), δ 4.2-6.7(*br*, 2H); MS, *m/e* 157 (M^+, 65%). **8**: MS, 177 (M^+ + 2, 6%), 175 (M^+, 17%).

(b) At 60°C. The same procedure as in (a) was applied to get **7** (0.730 g, 93%) almost free of **8**.

Kinetic Measurement-Action Spectra. To the solution of **6** (0.097 g, 0.5 mmol) in DMSO (20 m*l*) at 60 °C was added KO_2 (0.106 g, 1.5 mmol). The temperature of the reaction mixture was maintained at 60 °C throughout the whole measurements. At time *t*, an aliquot (1.0 m*l*) was withdrawn from the solution and diluted with water to 100 m*l* for the measurement of optical density. In the reaction with KOH, the same procedure was employed under the same conditions.

Results and Discussion

The results from the reaction of 2-chloropyrimidine (**1**) and 5-bromopyrimidine (**2**) with either KO_2 or KOH under several different conditions are summarized in Table 1. It can be seen in Table 1 that the reactions are too slow for any product(s) to be isolated at room temperature (15 °C). At a temperature of 60 °C, both **1** and **2** are transformed slowly to give product(s). In the case of **1**, corresponding hydroxypyrimidine (**3**) was obtained (equation 9).

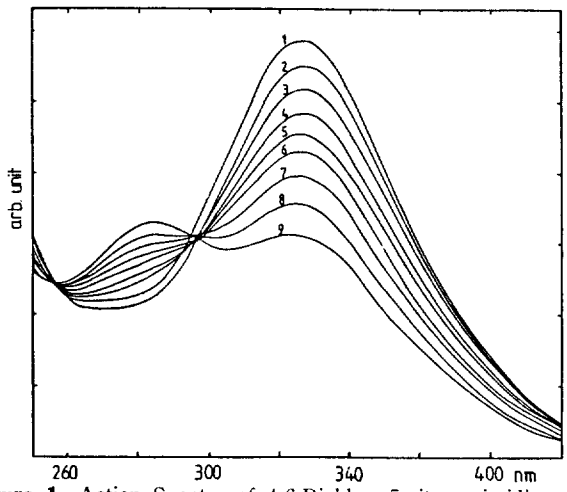
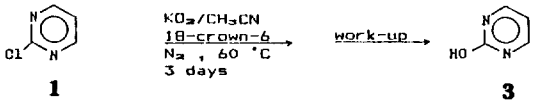
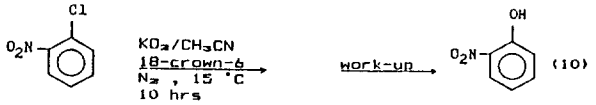


Figure 1. Action Spectra of 4,6-Dichloro-5-nitropyrimidine with KO_2 in DMSO at 60 °C: Curve 1; 10 hours, 2; 8", 3; 7", 4; 6", 5; 4.5", 6; 3.5", 7; 2.5", 8; 1.5", 9; 0.5" of reaction time.



1 **3**

Considering the significant electron deficiency at C-2, C-4, and C-6 but only relatively minor deficiency at C-5 in the pyrimidine ring[13], the low reactivity of **2** might be understood. In this regards low reactivity of **1** was puzzling since **1** was expected to react readily with KO_2 as 2-chloro-3-nitropyridine (**4**). As a matter of fact, 2-chloronitrobenzene (**5**) whose reactivity is apparently smaller than that of **4**[16] was found to react with KO_2 completely under our conditions (equation 10).



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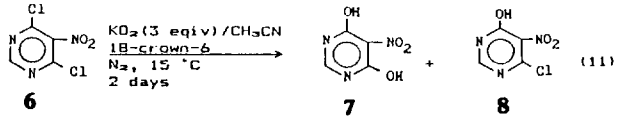
The results of equation 10 as well as those in Table 1 provide an example that halopyrimidines are less reactive than halonitrobenzene in reaction with superoxide.

We then next turned to the much more reactive 4,6-dichloro-5-nitropyrimidine (**6**). Reaction of **6** with KO_2/KOH proceeded readily as expected. As shown in equation 11 the reaction of **6** with KO_2 gave exclusively dihydroxylated product (**7**) together with monohydroxylated one (**8**) in a trace amount (equation 11).



6 **7** (80 %) **8** (trace)

The results from the reaction of **6** with KO_2/KOH are summarized in Table 2. As seen in Table 2, there is no difference in reactivity between superoxide and hydroxide in reaction with **6** under anhydrous acetonitrile media.

In order to exclude the possibility that the reaction with KO_2 be due to KOH, we carried out the separate experiments in carefully dried DMSO under the same conditions. Action

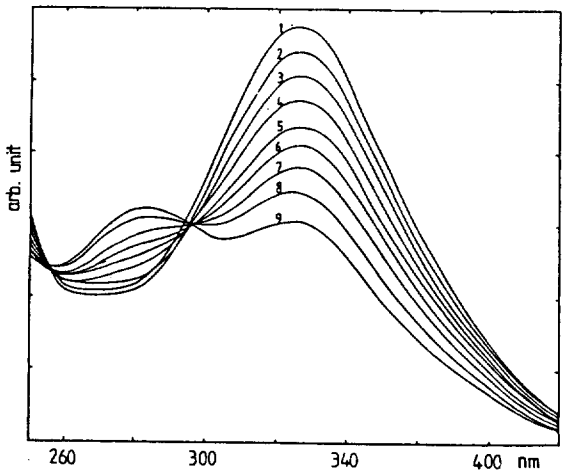


Figure 2. Action Spectra of 4,6-Dichloro-5-nitropyrimidine with KOH in DMSO at 60 °C: Curve 1; 10 hours, 2; 8″, 3; 7″, 4; 6″, 5; 4.5″, 6; 3.5″, 7; 2.5″, 8; 1.5″, 9; 0.5″of reaction time.

spectra, Figure 1 and 2, where curves of the same number represent the same time interval from the beginning of the reaction were obtained using KO_2 and KOH respectively. In Figure 1 and 2 it is seen that the peak at 325 nm (λ_{max} of **7**[17]) gradually grows up at the expense of the peak at 285 nm (λ_{max} of **8**[18]). These changes unambiguously come from the intermediacy of **8** in the formation of **7**. In addition to this from the optical densities of the same numbered curves, reactivity of superoxide is found to be almost equal to that of hydroxide again, well corroborating with the results in Table 2.

Then this result provides information that the reaction pathway between **6** and KO_2 might be S_NAr instead of $S_{RN}1$.

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