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REACTIONS OF 2-PHENYL-4,4-DIMETHYL-2-OXAZOLIN-5-ONE AND 2-PHENYL-4-ETHYL-2-OXAZOLIN-5-ONE WITH KO_2 IN APROTIC SOLVENTS (Issued as AECL No. 7964)

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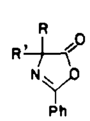
Abstract - The reactions of 2-phenyl-4,4-dimethyl-2-oxazolin-5-one (I) and 2-phenyl-4-ethyl-2-oxazolin-5-one (II) with KO_2 in tetrahydrofuran and freon are studied. Superoxide reacts with I to yield the N-benzoyl-α-aminoacid ring-opening product, indicating that O_2^- produces a nucleophilic attack at the carbonyl group of the oxazolinone. The oxazolinone II yields, in addition to the N-benzoyl-α-amino derivative, N-propanoyl benzamide (III) as the main reaction product. The results strongly suggest that III is formed after O_2^- has abstracted a proton from II, and then, species resulting from secondary reactions, such as oxygen, attack II to yield the final products. Several mechanistic pathways are discussed.

It is recognized that superoxide anion, (O_2^-) participates in important biochemical processes under both normal and pathological conditions (1,2); however, the mechanisms by which it acts in each of these instances are unclear. Consequently, a better understanding is needed of the reactivity properties of O_2^-.

Structurally, O_2^- may be considered a free radical, a base, a nucleophile and a complexing agent (3,4) and, depending on the reaction characteristics and conditions, it reacts according to these structural characteristics. Thus, one-electron transfer reactions are observed between O_2^- and quinones (5), peroxides and hydroperoxides (6), catechols, hydroquinones and ene-1,2-diols (7), vitamin E and related compounds (8), some unsaturated compounds (9) and certain aromatic derivatives (10). In water, O_2^- is a base with a strength comparable to that of acetate (11); however, in several non-aqueous media, its basicity is much greater than that of carboxylates (4), and it can induce proton-abstraction processes (12). Also, O_2^- participates in a number of nucleophilic displacements and complexing reactions (13-15). Although these modes of reaction may be expected, it is difficult to predict which one will predominate for a given set of conditions.

An approach that may help to better understand the behaviour of O_2^- would be to analyse its reactivity towards substrates that provide more than one mode of reaction. In this paper, we use this approach and report our results for the reactions of O_2^- with two 2-oxazolin-5-one derivates. The results show that, while the 2-phenyl-4,4-dimethyl-2-oxazolin-5-one (I) reacts with O_2^- at the carbonyl carbon to yield the corresponding ring-opening product, the homologue 2-phenyl-4-ethyl-2-oxazolin-5-one (II) reacts mainly in a different fashion, yielding CO_2 and an imide derivative. Several mechanistic possibilities are discussed.



I R' = R = Me

II R' = H, R = Et

MATERIALS AND METHODS

Synthesis of I and II

Oxazolinones I and II were obtained from the corresponding N-benzoyl-α-aminoacid derivative by refluxing it in acetic anhydride, as previously described (16). N-benzoyl-α-amino-isobutyric and N-benzoyl-α-aminobutyric acids were prepared from the corresponding amino-acid after reaction with benzoylchloride (16).

Synthesis of III

N-propanoyl-benzamide was prepared by refluxing benzamide and propanoic acid anhydride in the presence of catalytic quantities of concentrated H_2SO_4 (17). The product was isolated after adding an ice-water mixture and filtering off the white crystals (mp = 103°C). The NMR and mass spectra data are given below. NMR in $DMSOd_6$ with TMS; ppm (multiplicity): 8.9(t);7.9(q);2.4(m);1.9(m). MASS SPECTRA; m/e (relative intensity): 177(1);148(0.2);120(0.5);105(2.0);77(1.3).

Materials

Diphenylisobenzofuran (DPBF) (Aldrich), o-dibenzoylbenzene (o-DBB) (Aldrich), KO_2 (Alfa Prods), bicyclohexyl-18-crown-6-ether (Aldrich) and sodium methoxide (Aldrich) were obtained commercially. The solvents tetrahydrofuran (THF) (Fisher, HPLC grade) and freon (Dupont, E-4) were dried and freshly distilled before use. THF was pre-dried over CaH_2 (Fisher), then decanted and dried over $LiAlH_4$ and fractionally distilled under nitrogen.

Other solvents were HPLC grade and used without further treatment.

Analyses

The UV spectra were obtained in a Cary 15 spectrophotometer fitted with a temperature-controlled cell holder, an automatic scanning attachment and a magnetic stirrer. The temperature in the cell holder was controlled at 25°C by circulating water from a temperature-controlled bath. NMR spectra were obtained in a Perkin Elmer R-12 spectrometer. The HPLC system used was a Beckman model 334 with two model 110 pumps, a model 421 controller, a UV detector set at 254 nm and an Alteck RP8 reverse-phase column (10 μm; 25 cm long by 4.6 mm I.D.). In all cases, the column eluant was 10 mM $NH_4H_2PO_4$ (HPLC grade), pH 7.0, and acetonitrile in a 1:1 ratio, at a flow rate of 1 mL/min.

Reactions of I or II with water, ethanol and potassium-methoxide

These reactions were performed in a 10-mm UV cell, containing 3 mL of water, or methanol, and an aliquot of a stock solution of I or II in dry ether. Typically, the concentration of the stock solution was 5×10^{-3} M and aliquots of 30 μL of it were taken, so that the initial concentration of I or II in the cell was 5×10^{-5} M. In the case of the reactions with methoxide, 3 mL of methanol were placed in the cell; then aliquots of I, or II, and finally an aliquot of 1 to 10 μL of a 0.125 M sodium methoxide stock solution were added. The reactions were followed by repeat scanning between 210 and 300 nm at timed intervals.

These reactions were also studied by HPLC, by injecting aliquots from a reaction flask into the column and analysing and identifying the eluted fractions.

Reactions of I and II with KO_2

The reactions of oxazolinones I and II with KO_2 were studied in the UV. Mixtures of the corresponding oxazolinone solution in THF, or freon, crown ether and KO_2 were placed in a 10-mm UV cell and the reaction monitored by repeat scanning between 210 and 300 nm. A magnetic stirrer was used in the UV cell to help maintain the highest possible concentration of KO_2 in solution.

The analysis of these reactions by HPLC was performed by taking aliquots from the reaction vessel, protected from light, and injecting them into the HPLC column.

Similar techniques were used for reactions carried out in the presence of DPBF and for those in which the reaction mixture was saturated with N_2 or O_2.

Isolation and identification of III

The product III was isolated and identified from a reaction mixture of II in THF, or freon, to which crown ether and KO_2 (in excess) had been added. After 48 h, the reaction mixture was extracted several times with acetonitrile and the resulting solution was concentrated to about 1 mL. This solution was injected into the HPLC column. The appropriate fractions were collected and combined and the solvent was evaporated. Purification of the residue yielded white crystalline material with mp = 102°C. NMR and mass spectra were used to identify this substance as III, as well as to prove its correspondence with the synthetic product obtained by independent procedures (vide supra).

RESULTS AND DISCUSSION

The reactions of I and II with water, methanol and potassium methoxide were analysed by UV spectroscopy and HPLC, as described in the previous section. It was found that the correspondence between the two reactions is apparent, particularly from the similar absorption patterns and their isosbestic behaviour. Identification of the reaction products confirmed that these reactions are indeed identical since, in both cases, the N-benzoyl-α-aminoacid derivative is obtained. Thus, I and II undergo nucleophilic attack at C-5, followed by ring cleavage. This mode of reaction is also observed in the reaction of I and II with methanol and methoxide, where the N-benzoyl-α-aminoacid methyl esters are formed, and it agrees with previous studies on the reactivity of these types of oxazolinones with nucleophiles (16-18).

Figure 1a shows repeat scans in the UV region for the reaction of I with KO_2 in freon, which shows the same characteristics are observed for reactions of 2-oxazolin-5-ones with other nucleophiles. The product isolated from this reaction is N-benzoyl-α-amino-isobutyric acid, which would be formed after nucleophilic attack by O_2^- at C-5, followed by ring cleavage between positions 1 and 5. Then, further reaction of the intermediate with O_2^- yields the final product (15). Oxazolinone II, however, reacts with KO_2 in a different way. Figure 1b illustrates this reaction, as followed by repeat scanning in the UV region. Several differences from the pattern just discussed for I are observed: (a) there is no isosbestic behaviour in the process: (b) the absorption pattern of the product(s) shows two maxima rather than just one single absorption; and (c) this reaction occurs at significantly faster rate than the corresponding one for I, both in freon (ca. ten-fold rate increase) and in THF (ca. five-fold rate increase). These observations agree with a process in which parallel reactions take place and more than one product is formed.

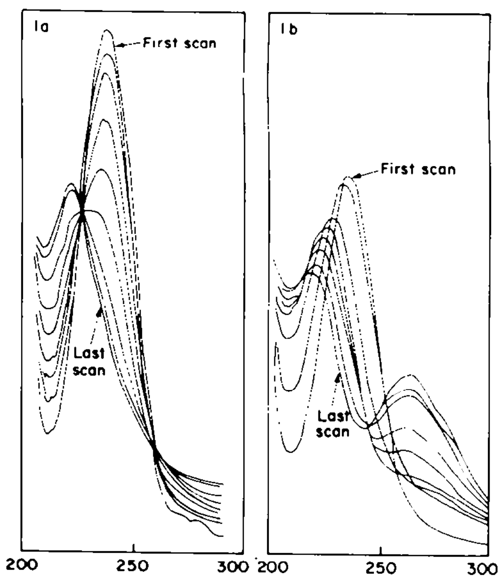


FIGURE 1. Kinetic runs for the reactions of KO_2 towards (a) oxazolinone I and (b) oxazolinone II followed by repeat scanning of the UV region between 210 and 300 nm.

The analysis of these reactions by HPLC shows that, while the reaction of I with O_2^- yields N-benzoyl-α-aminoisobutyric acid, the reaction of II forms two products: N-benzoyl-α-aminobutyric acid (ca. 10% yield in freon; ca. 25% yield in THF) and III (ca. 80% yield in freon; ca. 60% yield in THF). The latter compound was identified after its separation by HPLC and characterization by UV, NMR and mass spectroscopies. Also, III was prepared by an independent procedure, and this synthetic product was identical to that formed in the reaction of II with O_2^- (see Materials and Methods).

The formation of III might be explained by assuming that O_2^- reacts directly with II, as in the case of electron-poor olefins in aprotic solvents (19). It was proposed that O_2^- reacts with these olefins at one of the carbons of the double bond to form an endoperoxide, which breaks down to yield two ketones. Similarly, it may be thought that the enolic form of II could react with O_2^- producing an endoperoxide between positions 4 and 5 on the oxazolinone ring. This intermediate would rapidly yield CO_2 and III.

The direct reaction of O_2^- with II, discussed above, explains the observed products, but it does not take into account the strong basicity of O_2^- in aprotic media and the relatively acidic proton, formally at position 4 of II. In fact, careful analysis of reaction runs, such as that in Figure 1b, and consideration of the absorption characteristics of O_2^- and $HOO^\cdot$ strongly suggest that O_2^- (λ_{max} = 250 nm) first abstracts a proton from II to yield $HOO^\cdot$ (λ_{max} = 225 nm) (4). The rate of these proton abstraction reactions involving O_2^- varies and depends on the acidity of the proton abstracted. For instance, strong acids react very quickly with O_2^-, while weak acids, such as water in DMF, do so with a second order rate constant equal to $1 \times 10^{-3}\ M^{-1}\ s^{-1}$.

Once $HOO^\cdot$ has been formed, dismutation of O_2^- occurs, forming hydrogen peroxide and oxygen. The latter species could then react with II to yield the observed product. The process is represented in eqs 1 to 3 where OXA-H stands for II.

$$\text{OXA-H} + O_2^- \rightleftarrows HOO^\cdot + \text{OXA}^- \quad (1)$$

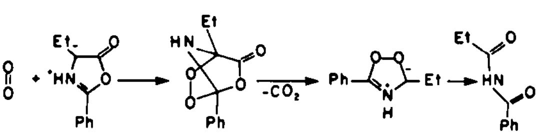
$$HOO^\cdot + O_2^- \rightarrow HOO^- + O_2 \quad (2)$$

$$OXA\text{-}H \text{ (or } OXA^-) + O_2 \rightarrow CO_2 + III \qquad (3)$$

This indirect mode of reaction of O_2^- towards acidic substrates in aprotic media has been observed previously and, in fact, the proton-induced dismutation of O_2^-, followed by the further oxidation of substrates by the dismutation products in these media, is one of its most important characteristics (4).

Probably, the most attractive mechanism for the reaction of II with oxygen is a 1,3-dipolar cycloaddition of oxygen to the oxazolinone ring, elimination of CO_2 and collapse of the resulting cyclic intermediate, to yield III. This type of cycloaddition has been well characterized for the reaction of oxazolinones that can form mesoinic compounds and dipolarophiles (20). In the presence of oxygen, III would be readily formed, as indicated in scheme 1.

Scheme 1



II III

There have been contradictory results in the literature for to the electronic state of the oxygen generated during non-enzymic dismutation of O_2^-. Some reports indicate that singlet oxygen is produced, others indicate that triplet oxygen is produced (4). We decided to investigate the nature of the oxygen formed in our reaction system. For this purpose, the reactions of I and II towards O_2^- in THF were analysed in the presence and absence of diphenylisobenzofuran (DPBF), a compound that has been used to detect the involvement of $^1O_2$ in a variety of reaction processes (21). The product of the reaction of DPBF with $^1O_2$ is o-dibenzoylbenzene (o-DBB).

Our results show that, when I reacts with O_2^- in the presence of DPBF, a small amount of o-DBB is formed, however, in the case of II, a much larger amount of this product is formed. As described in the previous section, these reactions are monitored at time intervals by injecting aliquots in the HPLC column and separating the oxazolinones, their reaction products, DPBF and o-DBB. The production of important amounts of o-DBB in the reaction of II with O_2^-, when DPBF is present indicates the involvement of $^1O_2$ in the system, however, since DPBF is not absolutely specific towards $^1O_2$, the result must be taken with caution.

Additional results suggesting the formation of $^1O_2$ were obtained from reactions of II with O_2^- in THF in which the medium was saturated with dry N_2 or dry O_2. There were no significant differences with respect to untreated medium with either of these gases, which again suggests the formation of $^1O_2$ during the reaction.

It is concluded that the reactions of I and II with O_2^- in aprotic solvents are good model systems to study different reactivity characteristics of O_2^-. When no acidic protons are present in the substrate, i.e. I, the reaction proceeds via nucleophilic attack of O_2^- at the carbonyl group, followed by a ring-opening process that yields an α-aminoacid derivative. When II reacts with O_2^-, III is formed in addition to the α-aminoacid derivative. The reaction is faster than in the case of I, and the proportion of products formed seems to depend on the nature of the solvent. The formation of III may be explained in several ways, one of which is a 1,3-dipolar cycloaddition of oxygen to II, followed by loss of CO_2. Finally, experiments with DPBF, and with N_2- and O_2-saturated solutions suggest that $^1O_2$ may be formed in this reaction system.

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