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SUPEROXIDE OXIDATION OF 1-NITROPYRENE-CIS-DIHYDRODIOLS

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ABSTRACT - KO_2 oxidation of cis-4,5-dihydro-4,5-dihydroxy-3-nitropyrene, 1 gives the known lactone, 3-nitro-5H-phenanthro[4,5-bcd]pyran-5-one, 2. Similarly the analogous 4,5-dihydrodiol, 3, gives the known lactone, 1-nitro-5H-phenanthro[4,5-bcd]pyran-5-one, 4. While the yield of 2 is 80%, it is only 16% for 4. A study of the latter oxidation, relying especially on the use of HPLC, led to a change in the conditions that increased the yield of 4 from 16 to 88%. The change was to quench the reaction with H_2O_2 shortly after it began instead of letting it proceed, as usual, for several hours before quenching with water.

Recently we reported that oxidation with potassium superoxide (KO_2) is useful for the preparation of aromatic 1,2-dicarboxylic acids from suitable precursors containing a dihydrodiol, ketone or quinone group[1]. This result is achieved by using conditions which achieve exhaustive oxidation of these compounds. While KO_2 had been established previously as a general purpose oxidizing agent[2,3], suitable precursors were never found to yield aromatic 1,2-dicarboxylic acids. This was due to a somewhat arbitrary selection of reaction conditions which limited the degree of oxidation[4,5]. KO_2 has also been used synthetically as a nucleophile[6]. While inherently it is a very weak oxidant (actually it is a mild reducing agent), it promotes oxidations by acting in conjunction with H_2O_2 (generated *in situ*) and O_2 (generated similarly and supplied by air)[2,3].

Here we extend our study of exhaustive KO_2 conditions by reacting two dihydrodiol derivatives of 1-nitropyrene, 1 and 3. For comparison, a corresponding dihydrodiol derivative of pyrene, 5, is oxidized as well.

Our interest in reacting dihydrodiols of 1-nitropyrene with KO_2 is largely motivated by the toxicological significance of such compounds. Nitropolyaromatics such as 1-nitropyrene account for some of the bacterial mutagenicity of emissions from diesel engines[7]. Dihydrodiol products can be formed as major metabolites of these nitropolyaromatics. Thus there is interest in the sensitive detection of these metabolites. The purpose of

subjecting such compounds to exhaustive KO_2 oxidation is to obtain products which are readily detected by gas chromatography, unlike the parent compounds.

EXPERIMENTAL

Materials

KO_2, 18-crown-6, dimethylformamide (DMF, HPLC grade), pyrene and 1-nitropyrene were obtained from Aldrich Chemical Co., Milwaukee, WI and used as received except for DMF which was dried over 40 nm molecular sieves. cis-4,5-Dihydro-4,5-dihydroxypyrene, 5, 4,5-pyrenedione, cis-4,5-dihydro-4,5-dihydroxy-3-nitropyrene, 1, 3-nitro-4,5-pyrenedione, 3-nitro-5H-phenanthro-[4,5-bcd]pyran-5-one, 2, cis-4,5-dihydro-4,5-dihydroxy- 1-nitropyrene, 3, 1-nitro-4,5-pyrenedione, 3a, and 1-nitro-5H-phenanthro[4,5-bcd]pyran-5-one, 4, were prepared as reported[7,8]. Authentic samples of 2 and 4 were also generously provided by Dr. Karam El-Bayoumy (American Health Foundation, Valhalla, NY).

Methods

^{1}H NMR were recorded with a Varian XL-300 spectrometer, and are referenced to tetramethylsilane. Electron impact mass spectra were obtained at 70 eV on a Nuclide 12-90G mass spectrometer. HPLC with detection at 254 nm was carried out on a Brownlee 4.6 x 220 mm RP-18, 5 um analytical column at a flow of 1 mL/min. Semipreparative separations were done on a Dynamax 10 x 250 mm RP-18, 5 um column at a flow of 4 mL/min. Solvent A was 0.1 M acetic acid (pH 2.9) and solvent B was acetonitrile. For all of the analyses, a gradient elution from 30 to 80% B in 20 min followed by an isocratic elution at 80% B for 10 min was used.

Oxidation with KO_2

a) Analytical Scale

Finely powdered potassium superoxide and 18-crown-6 (5/2 molar ratio of KO_2: crown ether) were suspended in dry DMF (0.5 mL). The substrate to be oxidized (3.6 umol) was dissolved in dry DMF (0.5 mL) and added dropwise to the KO_2 suspension, giving a mixture that was stirred vigorously in a stoppered amber vial for $\leq$ 2 h. An aliquot (0.10 mL) was removed, quenched with 0.40 mL H_2O, diluted with CH_3CN (0.50 mL), and 10 ul was subjected to reverse-phase HPLC. Yields were determined using authentic products to construct calibration curves.

b) Preparative Scale

This oxidation was performed on 20 mg of starting material (5 and 1), using, for 5, a 10-fold molar excess of KO_2 and a 4-fold molar excess of 18-crown-6 in 5 mL of DMF at RT for 2 h. For 1, a 4-fold excess of KO_2 and a 1.6-fold excess of 18-crown-6 was similarly used for 0.5 h. The reaction mixture was quenched with 5 mL of water and evaporated under reduced pressure. To the residue was added 5 mL of 3 M HCl. Three x 10 mL extractions with ethyl acetate were combined, dried (Na_2SO_4) and evaporated, yielding a solid that was dissolved in tetrahydrofuran and purified by semipreparative reverse-phase HPLC. The yield of 2 from 1 was 80% and the yield of 6 from 5 was 86%.

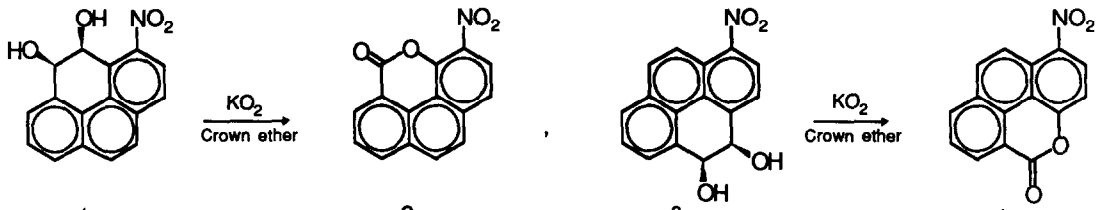
The products were confirmed by comparison (HPLC, mp and spectroscopic data) with authentic compounds, except in the case of 4,5-phenanthrenedicarboxylic acid. This latter compound was known, but an authentic sample was not available. Its structure was confirmed as follows: mp 258-260°C (lit.[9] mp 260°C); ^{1}H NMR (CD_3OD); δ 7.59 (t, 2H, $H_{2,7}$, $J_{1,2}=J_{2,3}$ $J_{6,7}=J_{7,8}$ =7.5 Hz), 7.79 (s, 2H, $H_{9,10}$), 7.98 (d, 2H, $H_{1,8}$), 8.02 (d, 2H, $H_{3,6}$); MS m/z (relative intensity) 248, M-18 (23), 220 (50), 204 (100), 176 (70), 163 (29), 150 (19).

c) Oxidation with KO_2/H_2O_2

Potassium superoxide (31 mg, 440 umol) and 18-crown-6 (11.5 mg, 44 umol) were added to a solution of 3 (260 ug, 0.9 umol) in 50 ul of DMF. The reaction was stirred at room temperature for 5 min, treated with 15.7 ul of 30% H_2O_2, and stirred for 2 h more. Ten microliters was removed, diluted 1:1 with 50% water/acetonitrile, and half was injected into an HPLC. Yield of lactone 4 was 88% (determined using authentic product to construct calibration curve).

RESULTS AND DISCUSSION

When we oxidize cis-4,5-dihydro-4,5-dihydroxy-3-nitropyrene, 1, and cis-4,5-dihydro-4,5-dihydroxy-1-nitropyrene, 3, with KO_2 in the presence of crown ether, the corresponding lactones 2 and 4 are formed in 80% and 16% yield, respectively. These products were also observed before when 1 and 3 are oxidized with MnO_2/O_2[7].



1 2 3 4

We observed that both lactones 2 and 4 were detected at the femtomole level by gas chromatography with electron capture detection (data not shown), consistent with our interest in the measuring the parent compounds 1 and 2 in biological samples by relying on the above reactions. However, the low yield of 4 was an impediment, so we examined its formation in more detail.

KO_2 oxidation of cis-4,5-dihydro-4,5-dihydroxypyrene, 5, the non-nitro analog of 1 and 3, forms the corresponding 4,5-phenanthrenedicarboxylic acid, 6, in an 86% yield. This type of reaction was reported previously for the KO_2 oxidation of similar polyaromatic hydrocarbons[1,10]. Thus, not only does the nitro group in 1 and 3 drive the formation of the lactones 2 and 4, but the low yield of 4 from 3 is tied to the presence of the nitro group including its positional relationship to the diol moiety in 3.

We next resorted to the use of HPLC, along with variation in the reaction conditions, to study further the poor formation of 4 from 3. For all three reactions (1 → 2, 3 → 4, and 5 → 6), the corresponding o-quinone for each starting material was observed (HPLC) as an early, significant intermediate, and it was the only intermediate seen throughout the course of the reactions for 1 → 2 and 5 → 6. In contrast, several intermediates (shortly to be discussed in more detail) were observed in the slower KO_2 reaction of 3. Authentic samples of the two o-quinones formed from 1 and 5 reacted under the same conditions to give products 2 and 6 in an 88% yield in a shorter time (30 and 1 minute, respectively). For the reaction of 3, this indicated that the low yield of 4 probably arises from events that take place after the formation of the quinone intermediate.

The course of the conversion of 3 to 4 (starting materials, intermediates and products as a function of reaction time), as revealed by HPLC, is shown in

Fig. 1a-d. After a very short reaction time (t = 1 second then quench with water; Fig. 1a), quinone 3a is the major intermediate and lactone 4 is very minor. Aside from a small amount of residual starting material, 3, two other, unknown intermediates are seen, 3b and 3c. Extending the reaction time to 30 minutes gives the chromatogram shown in Fig. 1b. The relative amounts of the compounds have changed, and an additional intermediate, 3d, is observed. Attempted isolation of 3b (collection and reinjection of its HPLC peak) gives the chromatogram shown in Fig. 1c in which peaks for 3b, 3c and 4 are present. Thus 3c and 4 arise from 3b. Similar collection and reinjection of 3c gives peaks for 3c and 4 (data not shown), and likewise collected/ reinjected 3d gives 3d and 4 (Fig. 1d). Clearly 3b, 3c and 3d are unstable intermediates enroute to 4.

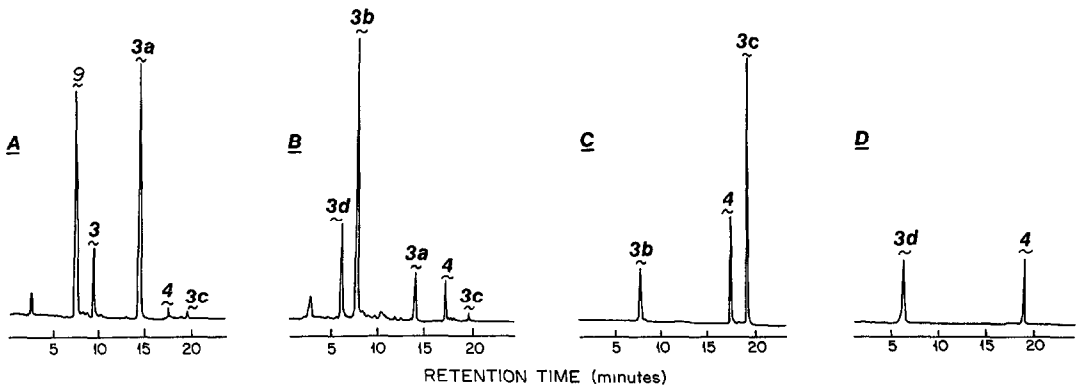
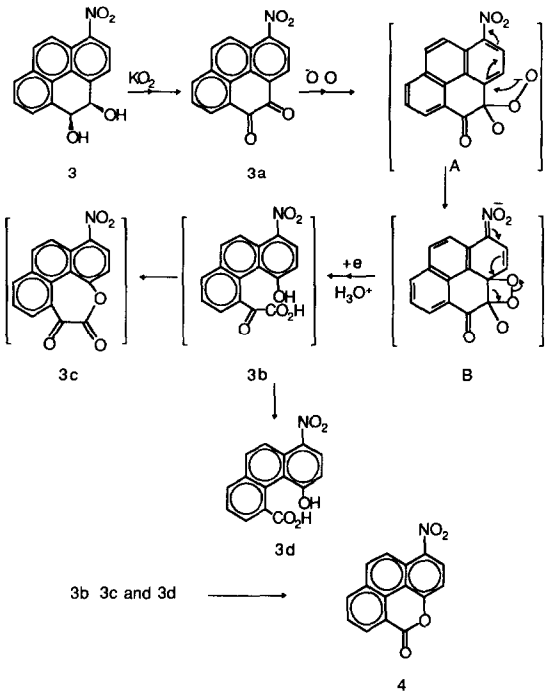


Fig. 1 HPLC chromatograms: (a) reaction mixture of 3 (20 mg) with a 4-fold molar excess of KO_2 and a 1.6-fold molar excess of 18-crown-6 in 5 ml of DMF after an aliquot was quenched with H_2O at t = 1 sec; (b) same as (a) but t = 30 min; (c) after attempted isolation (see text) of 3b; (d) after attempted isolation of 3d.

The HPLC results of Fig. 1 are not directly helpful in explaining the low yield (16%) of 4 from 3, since the degree to which the intermediates (3a-d) eventually form 4 is not defined. Further, additional side products may be present which escape detection by HPLC. Indeed, when the reaction is allowed to proceed on a preparative scale for more than 2 hours, an insoluble, brown precipitate forms, presumably polymeric side products. Since anion radical intermediates tend to develop in KO_2 oxidations of aromatic organic substrates[11], the apparent polymerization reaction is not surprising. The low yield of 4 from 3 therefore may arise because anion radical or other intermediates participate in reactions leading to polymers.

A likely structure for intermediate 3d is 4-hydroxy-1-nitrophenanthrene-5-carboxylic acid. We came to this conclusion by showing that 4 reversibly forms 3d (5 to 10% by HPLC) when subjected to alkaline hydrolysis.

Taking into account the above data, and what is known mechanistically about KO_2 oxidations[1,12,13], we postulate the steps and intermediates shown in Scheme 1 for the conversion of 3 to 4, aside from formation of polymer. For the diketone intermediate 3a, the carbonyl group para to the nitro group is expected to be the most reactive site for the nucleophilic attack by $O_2^{\cdot-}$, followed by intramolecular electron transfer to give intermediate A. A subsequent, intramolecular nucleophilic attack may take place as shown for A, promoted by the para (in 3) or ortho (in 1) nitro group. Subsequent intermediate B could then rearrange, as proposed, to give the postulated hydroxy, α-keto acid 3b (reversed phase HPLC shows that 3b is more polar than 3a). This acid, in turn, could cyclize to form the less polar α-ketolactone postulated for 3c. Two kinds of data support the structure proposed for 3c. First, the mass spectrum of 3c gives a molecular ion at m/z 293. Second, HPLC shows that 3c is less polar than 3b. Scheme 1 is also consistent with the observed sequence in which the HPLC peaks rise (and fall) during the course of the reaction. A similar mechanism would account for the formation of lactone 2 from compound 1. For 5, which lacks a nitro group, the corresponding intermediate A would more likely undergo intramolecular attack by the oxygen anion onto the second carbonyl grou followed by cleavage to form diacid 6.



Scheme 1

Although our HPLC assessment of the reaction of 3 with KO_2 did not reveal the polymeric side products, it did show that 3 forms the initial products 3a an 3b. As discussed above, we believe that 3b is an α-ketoacid. According to the literature, α-ketoacids can be oxidatively decarboxylated under basic conditions by hydrogen peroxide[14]. This led us to quench the reaction with H_2O_2 shortly after it began, which increased the yield of 4 from 16 to 88%.

In conclusion, reaction conditions and mechanistic insights have been achieved for the KO_2 conversion of diols 1 and 3 to the corresponding lactones 2 and 4 in high yield.

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