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Crystalline Diethylstilbestrol (DES) Quinone: Synthesis, X-ray Analysis, and Stability

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Diethylstilbestrol (DES) is a synthetic estrogen that has been shown to be carcinogenic in humans and animals.[1] While the exact mechanisms underlying DES carcinogenicity are not fully understood, it has been proposed that the oxidation of DES to the corresponding DES quinone by either peroxidase[2,3] or cytochrome P-450[4] may be responsible for its carcinogenic activity.[5−7] The existence of DES quinone (DES Q) has been inferred from its in vitro[2−4] and in vivo formation.[8] The synthesis of DES Q has been reported previously,[3,9] but these workers were unable to obtain DES Q in crystalline form. We report here a procedure for obtaining pure crystalline DES Q and present the results of spectroscopic and crystallographic analysis of this compound. Crystalline DES Q is stable at −70 °C but rearranges at room temperature to (*Z*,*Z*)-dienestrol (DIES); the crystal structure analysis reveals a close intermolecular approach between an oxygen atom and a methylene group that may be directly involved in this process.

Manganese dioxide (160 mg) or silver oxide (320 mg) was added to 80 mg of a magnetically stirred solution of DES in CH_2Cl_2 (40 mL), and the mixture was stirred at room temperature.[3] The oxidation with MnO_2 was complete (checked by TLC) within 10 min, whereas oxidation with Ag_2O required an additional 10 min. The reaction mixture was centrifuged at 1000*g* for 10 min to precipitate MnO_2 or Ag_2O, and the supernatant was filtered over Celite. To the yellow solution was added 40 mL of hexane, and the resulting solution was evaporated under vacuum at room temperature. A turbid solution was produced; subsequently a gummy precipitate adhered to the bottom of the flask. Continued evaporation resulted in partial clarification of the solution followed by turbidity and finally a clear yellow solution. This solution was pipetted out, and within a short period (usually 5−10 min) DES Q precipitated from this solution as a lemon yellow crystalline solid: mp 115−116 °C (dec); IR (KBr) 2980, 2900, 2880, 1635, 1627, 1173, 865 cm^{-1}; UV (CH_2Cl_2) λ_{max} 296, 335 nm, (CH_3OH) λ_{max} 203, 298, 335 nm, (H_2O) λ_{max} 311, 346 nm; ^{1}H-NMR ($CDCl_3$) δ 1.18 (t, 6H CH_3), 2.52 (m, 2H, CH_2), 3.04 (m, 2H, CH_2), 6.32 (dd, 2H, =CHC=O), 6.52 (dd, 2H, =CHC=O), 6.95 (dd, 2H, *CH*=CHC=O) 7.65 (dd, 2H, *CH*=CHC=O); MS (EI) *m*/*e* 268 (M + 2, 46), 266 (M$^+$, 100), 251 (40), 237 (45), 210 (13), 159 (8), 145 (27), 121 (21), 107 (23), 77 (9). High-resolution MS (EI) calcd for $C_{18}H_{18}O_2$ 266.3390; found 266.1326. The gummy precipitate formed during evaporation was found by TLC to be a mixture of DES Q and DIES.

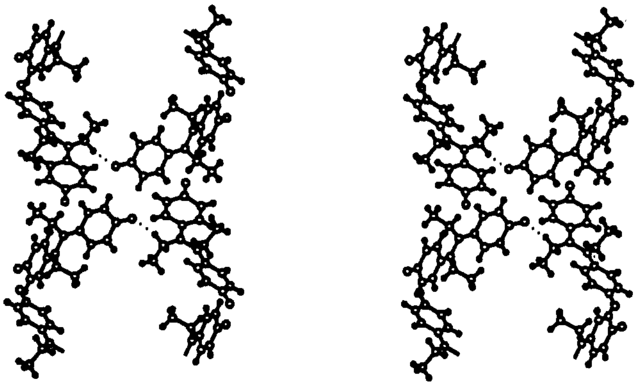

Figure 1. PLUTO[14] stereoview of the crystalline environment of O(1), dotted lines showing two examples of the close approach of O(1) to the C(2A) methylene group. To improve clarity, only portions of some of the molecules have been included in the figure.

Because the bright yellow, plate-shaped crystals of DES Q were very thin (maximum thickness approximately 0.02 mm) and transformed within minutes at room temperature, obtaining one suitable for X-ray diffraction studies was difficult. The crystal ultimately used for the structure determination was thin (0.02 mm) but was the thickest yet found. It was chosen from a batch of freshly-prepared crystals that had been removed from refrigeration at −70 °C only a few minutes previously. For the X-ray structure determination it was quickly glued to a glass fiber and immediately placed in the −100 °C nitrogen gas stream of a Rigaku AFC6S diffractometer equipped with a crystal cooling system.[10]

Although efforts to obtain the crystal structure were ultimately successful, the overall quality of the structure was adversely affected by the thinness of the crystal, which was responsible for a low data:parameter ratio of approximately 4:1. Bond lengths and bond angles found for the DES Q molecule in this structure are reasonable, but certain factors (such as some discrepancies between the molecules in analogous bond lengths) indicate that these results should be viewed cautiously. The molecule in the solid state assumes two independent but similar conformations, each molecule lying on a crystallographic 2-fold axis bisecting the central C−C bond. Both conformations are folded ones in which the quinone rings within each DES molecule are twisted well out of coplanarity with each other by torsional angles of −72(2)° (first conformation) and 75(1)° (second conformation) about the central C−C single bond. The folded shape of the molecule can be seen in Figure 1, which also shows (dotted lines) a close approach between O(1) and the C(2A) methylene group of a neighboring DES Q molecule. This particular approach involves the closest intermolecular O···H contact in the structure; no similar close interaction involving O(1A) (from the other independent molecule) is found. The distance between O(1) and the methylene carbon is 3.22(1) Å, and the distance between O(1) and the nearer of the two methylene hydrogens is approximately 2.4 Å. The (carbonyl

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(10) Crystals of DES Q are orthorhombic, space group *Pccn*, with a = 15.123(4) Å, b = 15.177(4) Å, c = 12.906(3) Å; V = 2962(2) Å^3; Z = 8. Of 6135 measured reflections, 750 were observed ($I > 3\sigma(I)$). Final R values were $R = 0.055$, $R_w = 0.049$. Further details of the structure analysis are available as supplementary material.

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C)—(carbonyl O)···(methylene H) angle is 135.7(6)°C. Because of the molecule's 2-fold symmetry, an identical interaction occurs at its other carbonyl group as well.

Studies in our laboratory have shown previously that activated MnO_2 is a good reagent for oxidizing catechols to *o*-quinones.[11] We utilized this approach to oxidize DES quantitatively (as determined by TLC analysis). Successful isolation of pure crystalline DES Q requires care in manipulating the CH_2Cl_2 solution following oxidation of DES. It is critically important that the crystalline material be filtered from the mixture immediately and stored at −40 °C or lower; otherwise the compound rearranges to DIES in the solid state. Attempts to grow crystals for X-ray studies are hampered by the fact that DES Q isomerizes to DIES and another product (see below). The degree of transformation can be noted by observing the change in color of pure crystalline DES Q from lemon yellow to yellow-orange and finally to amber (within 48 h).

UV and ^{1}H-NMR data we report here are similar to those reported earlier.[3,12] However, our ^{1}H-NMR results are slightly different from those reported by Siegle *et al.*[12] presumably because we used a higher field NMR spectrometer. Previous studies by Liehr *et al.*[3] showed that the mass spectrum of DES Q in solution was identical with that of DIES. These investigators proposed that DES Q undergoes isomerization to DIES, possibly during evaporation in the instrument. The mass spectrum we obtain from crystalline DES Q shows a fragmentation pattern identical to that of DIES except for an additional M + 2 peak at 268 which is indicative and characteristic of quinonoid compounds.[13]

Crystalline DES Q was found to be stable for at least 6 weeks at −70 °C. However, at 4 °C or above, the compound undergoes isomerization initially to DIES and another product, but eventually only DIES remains. It is interesting that although *solid* DES Q undergoes isomerization at room temperature, a *solution* of DES Q in $CHCl_3$ was found to remain unchanged for at least 3 days at room temperature, consistent with the results obtained by Liehr *et al.*[3] A time-course NMR analysis showed that a sample of pure crystalline DES Q was transformed within the first hour at room temperature to a mixture of DES Q (90%) and the other product, the one presumed from the mass spectroscopic results to have a quinonoid structure. At this point no DIES was observed. After 8−10 h at room temperature a mixture of DES Q, DIES, and the quinonoid compound was found. The NMR spectrum of solid DES Q after 24 h at room temperature showed the presence of DES Q, the quinonoid compound, and a considerable amount of DIES (Figure 2). Crystalline DES Q is transformed to DIES within 72 h. This transformation may be facilitated by the close (methylene H)···(carbonyl O) contact described above. This H atom is already in position for facile transfer to the carbonyl oxygen, and bond migration would lead to formation of the half-quinone (Scheme 1). Similar hydrogen transfer to the other quinonoid group in the phenol−quinonoid intermediate would lead to formation of DIES. Thus far, attempts to demonstrate the formation of the half-quinone intermediate in a solution of DES Q in $CHCl_3$ have not been successful. The fact that the DES Q molecules are dispersed in solution would make H transfer difficult and would explain the stability of DES Q in solution.

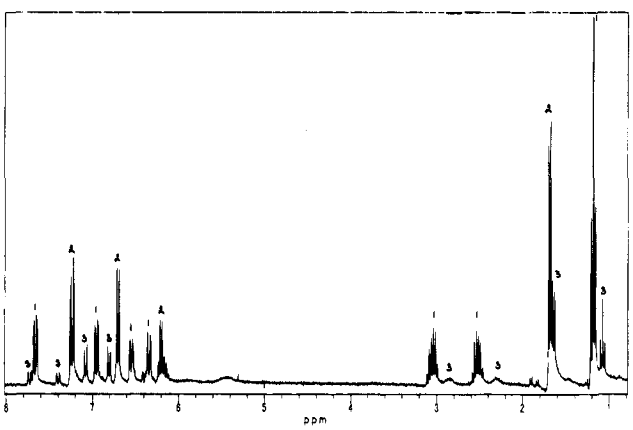
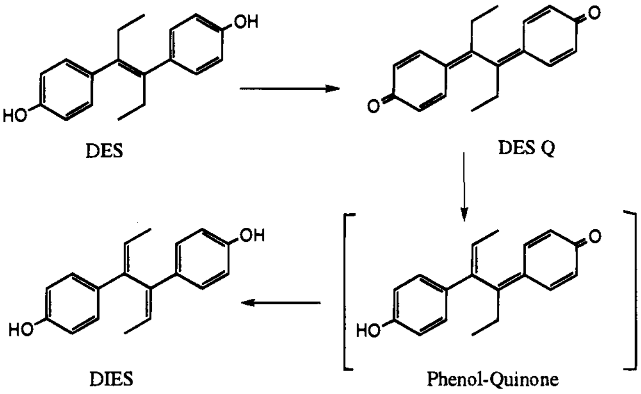


Figure 2. ^{1}H-NMR of DES Q after standing at room temperature for 24 h. Peak identification: 1 = DES Q, 2 = DIES, 3 = phenol−quinone intermediate.

Scheme 1. Synthesis and Rearrangement of DES Quinone



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Supplementary Material Available: Details of the data collection and structure solution and refinement, fractional atomic coordinates and equivalent isotropic thermal parameters, anisotropic thermal parameters, bond lengths and angles, torsional angles, intermolecular distances, least-squares planes, and ORTEPII figures (17 pages); observed and calculated structure factors (19 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, can be ordered from the ACS, and can be downloaded from the Internet; see any current masthead page for ordering information and Internet access instructions.

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