CONVERSION OF 2,5-DIMETHYLFURAN TO 2-HYDROXY-5-HYDROPEROXY-2,5-DIMETHYLDIHYDROFURAN, A TRUE $^1O_2$-DERIVED REACTION IN AQUEOUS $^1O_2$ GENERATING SYSTEMS

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Summary: It has been studied whether 2,5-dimethylfuran (DMF) is a specific $^1O_2$ trapping agent in aqueous system. The exposure of DMF to aqueous $^1O_2$ generating system (Rose Bengal photooxygenation system) gave 2-hydroxy-5-hydroperoxy-2,5-dimethyldihydrofuran (a hydrated form of endoperoxide, $^1O_2$-derived reaction product) and cis-diacetylethylene (cis-DAE), while the bromine-catalyzed autoxidation of DMF afforded only trans-DAE. In Fenton system (·OH generating system) DMF was converted in the main to cis-DAE, but not to the hydrated form of endoperoxide. The exposure of DMF to acetaldehyde-xanthine oxidase system failed to detect the hydrated form of endoperoxide, but chiefly yielded a non-specific oxidation product, cis-DAE.

Recently, Kellogg and Fridovich (1) have reported that when 2,5-dimethylfuran (DMF), a powerful $^1O_2$ trapping agent, was exposed to the acetaldehyde-xanthine oxidase system, a product (apparently identical with that obtained by the exposure of DMF to a sensitized photooxygenation system) was produced. Furthermore, their findings that the production of this unidentified compound was suppressed by the addition of catalase or superoxide dismutase support the hypothesis that oxidation of DMF may involve the generation of $^1O_2$ in the following reaction:

$$O_2^- + H_2O_2 \longrightarrow \cdot OH + {}^-OH + {}^1O_2$$

On the other hand, it has been clearly demonstrated that the

Abbreviations used: $^1O_2$, singlet molecular oxygen; NMR, nuclear magnetic resonance; IR, infrared.

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reaction of DMF with $^1O_2$ in methanol gave 2-methoxy-5-hydroperoxy-2,5-dimethyldihydrofuran (2). Therefore, the exposure of DMF to aqueous $^1O_2$ generating systems should give a hydrated form of the endoperoxide, 2-hydroxy-5-hydroperoxy-2,5-dimethyldihydrofuran as a common product. The present work was undertaken to test the conversion of DMF to the hydrated endoperoxide with $^1O_2$, ·OH or another oxidant in aqueous media, using non-enzymatic and enzymatic systems.

RESULTS AND DISCUSSION

As pointed out by several workers (2,3) the first product being formed from DMF by the $^1O_2$-pathway should be an endoperoxide. This compound, however, would not be stable enough to isolate it in pure state under usual conditions (3) but could be converted to its stable form in a certain solvent; i.e. 2-methoxy-5-hydroperoxy-2,5-dimethyldihydrofuran in methanol (2) and 2-hydroxy-5-hydroperoxy-2,5-dimethyldihydrofuran (the hydrated endoperoxide) in water as described here under "Experimental Section".

Even though the hydrated endoperoxide is easily polymerized by its autoxidation (4), part of this compound could be isolated as crystals or detected by TLC followed by spraying NaI in acetic acid. To confirm this, DMF was exposed to a known $^1O_2$ generating system (Rose Bengal-sensitized photooxygenation system) or autoxidation systems (·OH generating system and Br_2 system) and its products were identified by the procedures described under "Experimental Section".

As shown in Table I, the hydrated endoperoxide was detected as an oxidation product of DMF in Rose Bengal photooxidation system, but not in the autoxidation systems in which cis- or trans-1,2-diacetylethylene (DAE) was the main product. Even though cis-DAE could be derivedfrom an endoperoxide of DMF (a precursor of the

Table I. Reaction of DMF in $^1O_2$ Generating and Autoxidation Systems

Systems	Reaction Time (hr)	Ether Extractable Residue[a] (%)	Products: Hydrated Endoperoxide[c] (%)	cis-DAE[d] (%)	trans-DAE[e] (%)	DMF Recovered (%)
(P)-Rose Bengal Photooxygenation	10	17	0.67	2.1	0.0	62
Bromine Oxidation	1/2	43	0.0	0.0	8.9	37
Fenton System	2	-	0.0	1.5 (mixture)[g]		83

a. Weight(%) was based on the amount of DMF initially used.
b. Yield(%) was based on the amount of DMF initially used.
c. NMR spectrum of crystallized materials in dimethylsulfoxide-D_6 was coincided with that of authentic hydrated endoperoxide. R_f (TLC) was 0.07.
d. Oily material. NMR spectrum in CCl_4 had peaks at 2.25 (CH_3CO) and 6.17 (CH=CH); all were sharp singlets of relative area 3:1 respectively. IR spectrum was coincident with that of authentic sample. R_f (TLC) was 0.27.
e. NMR spectrum in CCl_4 had peaks at 2.32 δ (trans CH_3CO) and 6.71 δ (CH=CH); all were sharp singlets of relative area 3:1, respectively. IR spectrum was coincident with that of authentic sample. m.p.78 C. R_f (TLC) was 0.36.
f. Expressed as ratio to DMF in experimental vessel to that in control vessel (DMF in water-acetone or in buffer). DMF in both systems was determined, after the time cited in the Table, by gas phase chromatography (Benton 34 (10%) + DIDP on Celite 545, column length 2m, at 30 C).
g. Determined by gas phase chromatography: Carbowax 20M (10%): column length, 1m; temperature, 88°C. Judging from the darkness of the spots visualized by spraying 50% sulfuric acid followed by charring over a hot plate, formation of cis-DAE predominated over that of trans-DAE.

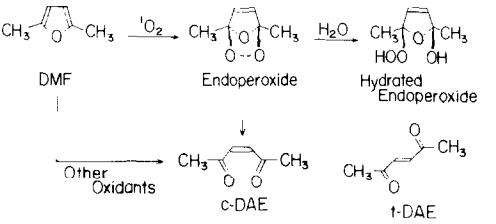

Figure 1

hydrated endoperoxide), this would not be a true $^1O_2$-derived product (Fig 1).

When DMF was exposed to the acetaldehyde-xanthine oxidase system, it was oxidized to cis-DAE, but not to the hydrated endoperoxide. Since this system is known to produce ·OH (5), the result obtained do not serve as evidence for the generation of $^1O_2$ in the system. Therefore, DMF, like 2,5-diphenylfuran (6), must be used with great care in the detection of $^1O_2$ generation in aqueous system.

EXPERIMENTAL SECTION

1) Preparation of trans- and cis-DAE - trans-DAE was prepared by the oxidation of acetonylacetone with selenious acid (7). cis-DAE was prepared from trans-DAE by the procedure identical with that employed for the synthesis of cis-dibenzoylethylene from its trans-isomer (8).
2) Preparation of 2-hydroxy-5-hydroperoxy-2,5-dimethyldihydrofuran This was prepared by the exposure of DMF to NaOCl-H_2O_2 system (a $^1O_2$ generating system). DMF (12.1 g, 0.126 mole) was dissolved in acetone (77ml), the solution was chilled, and 30% H_2O_2 (38 ml, 0.835 mole) was added. The resultant mixture was stirred vigorously at 4°C in a pyrex vessel and then in the dark NaOCl (210 ml, 0.285 mole) was introduced below the surface of liquid via a capillary tube. After 2.5 hours the reaction mixture was extracted three times with 1/3 volume of ether and combined extract was dried over Na_2SO_4. Removal of ether gave oily product (1.42 g), which eventually solidified after treatment with ether-benzene. Crystallization from hot ether yielded pure hydrated endoperoxide (containing 1 mole of H_2O), 0.2 g as colorless long plates with m.p. 110-112°C (uncorrected). Foote et al have synthesized this compound (but does not contain water) and reported to have m.p. 134-136°C (9).
$C_6H_{10}O_4 \cdot H_2O$ calculate: C 43.91, H 6.14, Found: C 43.71, H 6.04
The NMR spectrum in dimethylsulfoxide-D_6 had peaks at 1.45 δ (CH_3), 5.94 δ (CH=CH) and 11.4 δ (OOH and OH); all were sharp singlets of relative area 3:1:1, respectively. The NMR in D_2O showed peaks at 1.53 δ (CH_3) and 6.05 δ (CH=CH) without peak corresponding to OOH and OH groups. Thin layer chromatography

(silica gel G) of the compound irrigated with benzene-acetone (10:1 v/v) revealed single spot (R_f, 0.07) by spraying 50% sulfuric acid followed by charring over hot plate or NaI in acetic acid.

3) Rose Bengal photooxygenation and autoxidation systems
The (P)-Rose Bengal photooxygenation system was composed of 600 mg of Photox and 126 mmoles of DMF in 300 ml water-acetone mixture (5:1 v/v). The mixture was stirred vigorously at 10°c in a pyrex vessel and irradiated with 500 W tungsten lamp, bubbling with O_2. The bromine oxidation system contained 10 mmoles of DMF in 20 ml of acetone, 6.5 mmoles of Br_2, 0.25M potassium phosphate buffer (pH 7.8) in a total volume of 100ml. The final pH was dropped down to 6.8 at the time indicated (30 min). The modified Fenton system contained 2.5 mmoles of DMF in 25 ml of acetone, 1.3 mM $FeSO_4$, 6.3 mM EDTA, 14 mM ascorbate and 0.1 M sodium phosphate buffer (pH 7.8) in a total volume of 250 ml.

In each case, the reaction mixture was extracted three times with ether, pooled ether extract was dried over Na_2SO_4, and the ether was removed under reduced pressure. The residue in acetone was applied on a preparative thin layer plate (silica gel G) and chromatographed in benzene-acetone (10:1 v/v). The compounds on the plate were eluted with acetone. The hydrated endoperoxide and trans-DAE were further purified by crystallization. Characterization of the products were carried out as described in the legend to Table I.

4) Acetaldehyde-xanthine oxidase system - Freshly distilled DMF (576 mg, 6 mmoles) in 30 ml of acetone was added to 270 ml of the reaction mixture which consisted of 10 mM acetaldehyde, 0.1 mM EDTA, 50 mM potassium phosphate buffer (pH 7.8). The resultant mixture was incubated with xanthine oxidase (8.4 units) at 37°C in the dark with gentle agitation (standard incubation). After one hour the mixture was extracted three times with 1/2 volume of chloroform, the combined extract was dried over Na_2SO_4 and the organic solvent was concentrated to 0.5 ml under reduced pressure (chloroform extract). In some cases, the reaction mixture was chilled in dry ice-acetone mixture and liophilized, the resulting residue was extracted either with acetone or ether and the organic solvent was concentrated under reduced pressure (acetone or ether extract).

TLC of an aliquot of the chloroform extract gave a compound (A) with the same migration as that of authentic cis-DAE and two other minor components. To obtain (A) in quantities sufficient to study its properties, the chloroform extract obtained from each of three standard incubations was combined and (A) was isolated by the preparative TLC described in experiment 3. The identity of (A), eluted from the silica gel G plate with acetone, was established by Mass, NMR and IR spectral informations, using cis-DAE and trans-DAE as reference compounds. The Mass spectrum of (A) showed prominent ions of m/e 112 (M), 97 (M-15; loss of CH_3), identical with the spectra of cis-DAE and trans-DAE. The IR and NMR spectra of (A) showed a prominent peak at 1,690 cm^{-1} (characteristic of carbonyl group) in IR (CCl_4) and two singlets at 2.23 δ (CH_3) and 6.20 δ (CH=CH) showing integrated ratio of 3:1 in NMR (CCl_4), respectively. These values were identical with those obtained with cis-DAE. A possible isomerization of trans-DAE to cis-DAE during reaction and isolation was examined by replacement of DMF with trans-DAE in the standard reaction mixture and analysis of the products by the procedure identical with those employed for DMF. The results indicated that little if any isomerization of

trans-DAE had occurred under our experimental conditions. Unexpectedly, TLC of each of three kind of extracts (chloroform, acetone and ether) gave no detectable hydroperoxide corresponding to the hydrated endoperoxide when sprayed with NaI-CH_3COOH or by 50% H_2SO_4 followed by charring over hot plate. When authentic hydrated endoperoxide was exposed to the acetaldehyde-xanthine oxidase system which contained the same components as those in the standard reaction mixture except for the replacement of DMF with hydrated endoperoxide, it gave no detectable cis-DAE and trans-DAE on a silica gel G plate, indicating that the hydrated endoperoxide is not a precursor of cis-DAE in acetaldehyde-xanthine oxidase system.

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