Complex Formation between Menadione and Cetylethylmorpholinium Ethosulfate: Effect on UV Photodegradation of Menadione

C. R. KOWARSKI ˣ and H. I. GHANDI

Abstract □ The process of menadione photodegradation can be enhanced or diminished by other compounds. The presence of the quaternary ammonium compound cetylethylmorpholinium ethosulfate (I) in solutions of menadione was found to slow the rate of photodegradation by UV light (253.7 nm). The mechanism of this effect may be due to complex formation between menadione and I. Complex formation was demonstrated by a shift in the absorption peaks of menadione from 245 and 260 nm to 251.5 and 261.5 nm, respectively. The equilibrium constant of this complex was calculated to be 1.647 *M*.

Keyphrases □ Menadione—effect of quaternary ammonium compound on UV photodegradation rate, complex formation □ Photodegradation of menadione by UV light—effect of quaternary ammonium compound, complex formation □ Cetylethylmorpholinium ethosulfate—effect on UV photodegradation of menadione, complex formation

Menadione (2-methyl-1,4-naphthoquinone; vitamin K_3) is degraded by exposure to light. The photolysis of menadione is diminished in the presence of anions such as chloride and bromide (1–8) but not sulfate (9). Some surfactants such as polysorbate 80 diminish the photodegradation of menadione, but polyoxyl 40 stearate enhances it (10).

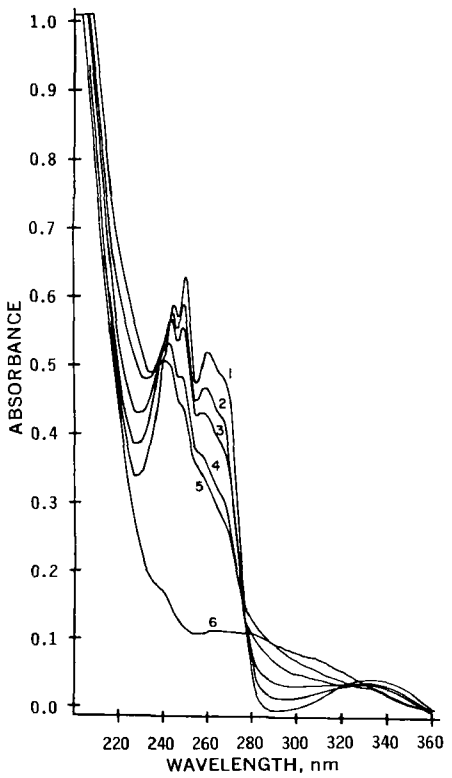

Figure 1—*Set of UV spectra obtained during photolysis of menadione. Key: 1, initial spectrum; 2, at 10 min; 3, at 20 min; 4, at 40 min; 5, at 60 min; and 6, at 3 hr from the beginning of irradiation.*

Table I—Composite Photochemical Results of Menadione in Solutions Containing Compound I

I Concentration, % (w/v)	K, $min^{-1} \times 10^{-3}$	K/K_{H_2O}, 0.2 *M* Borate Buffer
0	4.863	1.000
0.1	2.141	0.440
1.0	1.635	0.336
2.5	1.289	0.265
5.0	0.783	0.160

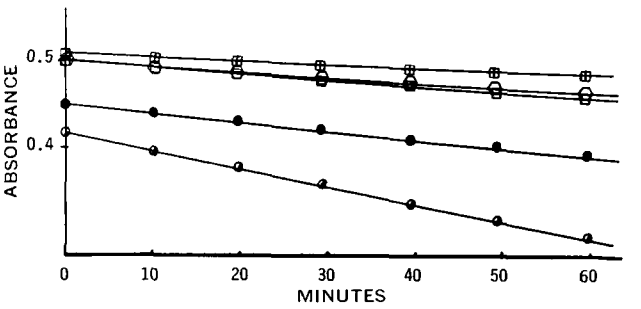

Figure 2—*Rates of photodegradation of menadione in aqueous solutions containing various concentrations of I as expressed by the change in absorbance at 250 nm with time. Key: □□, 5% I; ○, 2.5% I; □, 1% I; ●, 0.1% I; and ◑, water.*

The purpose of this study was to explore the effect of a quaternary ammonium compound on the photodegradation of menadione. Cetylethylmorpholinium ethosulfate (I) was chosen because this compound does not absorb UV light in the same region as menadione and because the sulfate ion does not have any effect on the photodegradation of menadione (9).

The naphthoquinone moiety present in menadione absorbs UV radiation over the 245–260-nm region. Therefore, photodegradation studies were carried out by irradiating with UV light at 254 nm.

EXPERIMENTAL

Materials—Menadione[1], mp 105°, and cetylethylmorpholinium ethosulfate[2] (I) were obtained commercially.

Photodegradation—The light source employed[3] was positioned at a distance of 60.0 mm from a quartz cell of 1 cm diameter. Fresh solutions of menadione in 0.2 *M* borate buffer solutions of pH 5.5 were placed in the sample cell. The absorption spectra[4] were determined during photolysis.

Interaction in Ground State—The solubility of menadione in

[1] K&K Laboratories, Plainview, N.Y.

[2] Atlas Chemical Industries, Wilmington, Del.

[3] A Pen-Ray mercury lamp, model SCT-1, black light (Eastern Corp.) with intensity of 2400 U W/cm² and 253.7 nm radiation.

[4] UV absorption was measured in a Cary model 15 double-beam scanning UV spectrophotometer and a Beckman DU spectrophotometer.

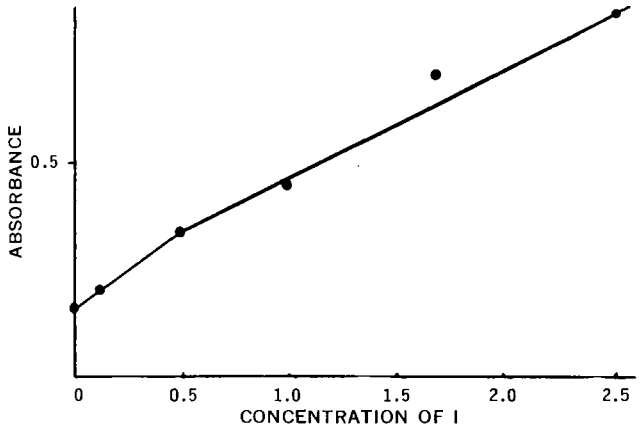

Figure 3—*Solubility of menadione in an aqueous solution as a function of the concentration of I (% w/v).*

the presence of I was studied by placing 100 mg of menadione in each of a series of 125-ml bottles containing 50 ml of 0.2 *M* borate buffer in which varying amounts of I were dissolved.

The complex determination was carried out by mixing varying concentrations of I with a constant concentration of menadione, 5.19×10^{-5} *M*, and recording the absorption spectra.

RESULTS

Kinetics of Menadione Photodegradation—The photodegradation of menadione solution was expressed as the rate of change of absorbance at 250 nm. The absorbance at this wavelength involves only the intact menadione (3, 6). A set of UV spectra obtained during photolysis is shown in Fig. 1. This figure demonstrates the gradual disappearance of the 250-nm absorption peak during photolysis. The effect of I on the rate of photodegradation was expressed by determining the apparent first-order rate constant, *K*, according to Eq. 1:

$$K = \frac{2.303}{t} \log \frac{C_0}{C} \qquad \text{(Eq. 1)}$$

For every rate study with I, a control study was run without it, and the ratio of the apparent first-order rate constants, K/K_{H_2O}, was determined (Table I and Fig. 2).

Interaction in Ground State—An increase in the solubility of menadione occurred in the presence of I, particularly when the concentration of I rose above 0.5% (w/v) (Fig. 3).

The presence of I also led to changes in the absorption spectrum of menadione. This phenomenon can be used to suggest the nature of this interaction. A maximum lowering of the 250-nm absorption peaks occurred when the concentration of I reached 2.78 *M*. At this concentration, the 245-nm absorption peak had shifted to 251.5 nm and the 260-nm absorption peak had shifted to 261.5 nm.

These shifts in the peaks, as well as the decrease in the absorbance of menadione by the addition of I, suggested a mechanism for

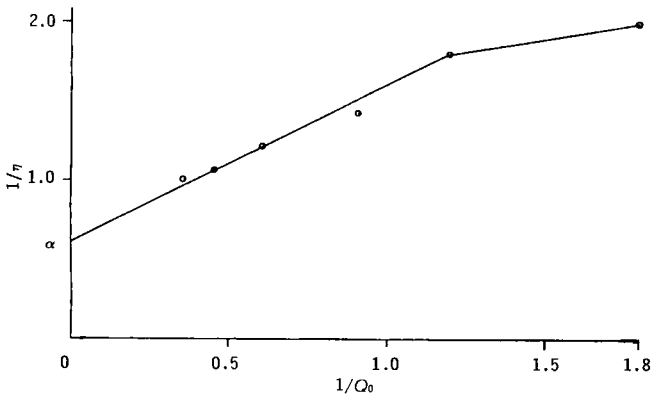

Figure 4—*Relationship between the concentration of I* [Q_0] *and the concentration of the complex* [η].

Table II—Relationship between the Concentration of Compound I [Q_0] and the Concentration of the Menadione–I Complex at 25° (Menadione Concentration = 5.19×10^{-5} *M*)

Q_0, *M*	Δi	$\frac{\Delta i}{\Delta s} = \eta$
0.5565	0.0630	2.0000
0.8347	0.0700	1.8001
1.1130	0.0880	1.4318
1.6696	0.1040	1.2116
2.2262	0.1190	1.0588
2.7825	0.1260	1.0000

the slowing down of the photodegradation of menadione. According to Kunio and Matsuoka (11), these two phenomena indicated the formation of a complex between menadione and I in the ground state.

The dissociation constant for the complex of I and menadione was calculated according to Kunio and Matsuoka (11) as follows:

$$\frac{\Delta i}{\Delta s} = \eta \qquad \text{(Eq. 2)}$$

where Δi = the difference between the absorbance of menadione in borate buffer and in various concentrations of I, and Δs = the difference between the absorbance of menadione in borate buffer and the maximal absorbance at the highest concentration of I. The absorbance was measured at 250 nm.

The quotient of the complex concentration [*MQ*] and the menadione concentration [*M*] is:

$$\beta = \frac{[MQ]}{[M]} \qquad \text{(Eq. 3)}$$

The proportionality factor is calculated with:

$$\beta = \alpha\eta \qquad \text{(Eq. 4)}$$

The equilibrium constant, *K*, is found by:

$$K = \frac{[M][Q]}{[MQ]} \qquad \text{(Eq. 5)}$$

where [*M*] and [*Q*] are the concentrations of menadione and I, respectively; and [*MQ*] is the concentration of the complex formed.

Since the concentration of the menadione is insignificant as compared to that of I, the amount of the I bound in the ground state is also insignificant as compared to the total concentration of I. Therefore, the concentration of $Q = Q_0$, $K = [(1 - \beta)/\beta]\,[Q_0]$, and Eq. 1 becomes:

$$K = \frac{1 - \alpha\eta}{\alpha\eta}\,[Q_0] \qquad \text{(Eq. 6)}$$

or:

$$\frac{1}{\eta} = \alpha K \frac{1}{[Q_0]} + \alpha \qquad \text{(Eq. 7)}$$

If $1/\eta$ *versus* $1/Q$ is plotted (as shown in Fig. 4), the intercept gives the value α. Thus, the equilibrium constant can be calculated. At a I concentration of 1.6696 *M*, the equilibrium constant was 1.647 *M* and the concentration of complex formed was 1.014×10^{-5} *M*.

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Synthesis and Evaluation of Antitumor Activity of 1-[*N*,*N*-Bis(2-chloroethyl)sulfamoylphenyl]-3,3-dialkyltriazenes

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Abstract □ Twenty-two 1-*p*-sulfamoylphenyl-3,3-dialkyltriazenes, 1-*p*-dialkylsulfamoylphenyl-3,3-dialkyltriazenes, and 1-*p*-[*N*,*N*-bis(2-chloroethyl)sulfamoylphenyl]-3,3-dialkyltriazenes were synthesized from their corresponding amines. Six of the compounds tested were devoid of antitumor activity.

Keyphrases □ Triazene derivatives—synthesis of 22 1-*p*-sulfamoylphenyl-, 1-*p*-dialkylsulfamoylphenyl-, and 1-*p*-[*N*,*N*-bis(2-chloroethyl)sulfamoylphenyl]-substituted 3,3-dialkyltriazenes, six compounds screened for antitumor activity □ Sulfamoylphenyltriazenes—synthesis of 22 derivatives, six screened for antitumor activity □ Antitumor activity—six of 22 synthesized sulfamoylphenyltriazenes screened

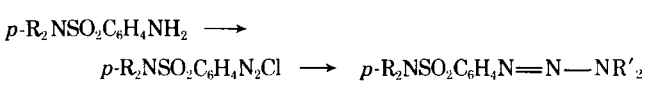
A number of 5-triazenoimidazoles with pronounced activity against experimental tumors were synthesized previously (1, 2). 5-(3,3-Dimethyl-1-triazeno)imidazole-4-carboxamide, in particular, is clinically useful for the induction of temporary remission in malignant melanoma (3). Some derivatives of phenyltriazenes have exhibited antitumor activity (4, 5), and some derivatives of N^1,N^1-bis(2-chloroethyl)-sulfanilamide were also found to be active against experimental tumors (6). Several *p*-sulfonyl- and *p*-sulfamoylcarbanilic acid esters were found to be inactive in mouse lymphoid leukemia (7, 8).

To extend these observations, it was decided to prepare compounds having *p*-sulfamoylphenyl, *p*-dialkylsulfamoylphenyl, and bis(2-chloroethyl)sulfamoylphenyl groups and the triazeno moiety in their molecules. Sulfanilamide, N^1,N^1-dialkylsulfanilamide, and N^1,N^1-bis(2-chloroethyl)sulfanilamide were readily converted to the desired triazeno derivatives by treating the appropriate diazotized amines with the secondary amines in sodium carbonate solution (Scheme I). All prepared compounds are summarized in Table I.

Compounds VI*a*, II*b*, V*b*, Ic, III*c*, and V*c* were tested *in vivo* for antitumor activity against mouse lymphoid leukemia L-1210, resulting in a T/C percent of 120 or less at 400 mg/kg (increase in survival of treated animals).

EXPERIMENTAL[1]

1 [*N*,*N*-Bis(2-chloroethyl)sulfamoylphenyl] 3,3-pentamethylenetriazene (IVc) was obtained from 2.97 g (0.01 mole) of N^1,N^1-bis(2-chloroethyl)sulfanilamide prepared according to Brintzinger *et al.* (9). The sulfanilamide derivative was added to a mixture of 25 g of crushed ice and 3.5 ml of concentrated hydrochloric acid with vigorous stirring at 0–5°. Diazotization was achieved by the slow addition, accompanied by thorough agitation, of sodium nitrite [0.7 g (0.01 mole) dissolved in 5 ml of water]. After standing at 0–5° for 20 min, the solution was filtered rapidly



Scheme I

[1] Melting points were measured on a Kofler hot-stage microscope and are uncorrected. The IR spectra were recorded on a Leitz model III spectrograph. NMR spectra were taken on a Varian A60A instrument. Mass spectra were recorded on a Varian Mat 111 spectrograph. UV spectra were obtained using a Varian-Techtron 635 recording instrument. All compounds were subjected to IR, NMR, and mass spectroscopy, and the results were as expected.