

volumetric flask at pH 7.0 and 7.5. Two solutions at pH 7.0 and 7.5 were equilibrated at a reaction temperature of 22.5°; the third solution at pH 7.0 was equilibrated at a reaction temperature of 37°. An aliquot was withdrawn periodically and filtered<sup>11</sup> (5- $\mu$ m pore size), and the maximum absorbance at  $\lambda$  275 nm was measured by running a UV spectrum from 400 to 260 nm. A plot of log concentration I remaining in solution versus time was constructed.

## RESULTS AND DISCUSSION

**Degradation Products**—This study shows that I undergoes degradation in an aqueous medium (pH 7.0 and 7.5). The degradation of I and the formation of the degradation products are depicted in Schemes I–III. The major degradation product isolated by chloroform extraction from the reaction was bis(2,6-dichlorobenzyl) disulfide (IV). The identity of this new compound, mp 107–108°, was confirmed by IR spectroscopy: 3060, 2950, 1580, 1439, 1090, 897, 885, 781, and 759  $\text{cm}^{-1}$ ; NMR (carbon tetrachloride plus tetramethylsilane):  $\delta$  4.3 ( $\text{CH}_2$ ) and 7.42 (aromaticity) ppm; and TLC, one spot visualized under UV light after fluorescein spray. In addition, a Raman spectrum<sup>12</sup> was obtained to confirm the S–S stretching vibration at 502  $\text{cm}^{-1}$  of IV (Fig. 1).

*Anal.*—Calc. for  $\text{C}_{14}\text{H}_{10}\text{Cl}_4\text{S}_2$ : C, 43.77; H, 2.62; Cl, 36.91; S, 16.69. Found: C, 43.56; H, 2.52; Cl, 37.37; S, 17.27.

The crystals that accumulated above the solution at pH 7.5 were identified as 2,6-dichlorobenzylthiol (II). The identity of this new product, mp 38°, was confirmed by IR spectroscopy (2550, 1580, 1560, 1437, 1090, 975, 775, and 755  $\text{cm}^{-1}$ ).

*Anal.*—Calc. for  $\text{C}_7\text{H}_6\text{Cl}_2\text{S}$ : C, 43.54; H, 3.13. Found: C, 43.43; H, 2.90.

This compound had a very disagreeable odor.

<sup>11</sup> Millipore.

<sup>12</sup> Model 82 laser-Raman spectrophotometer, Cary Instruments.

Cyanoguanidine (V) was isolated as the ethanol-soluble fraction and was shown to be comparable with the authentic compound by IR, melting point (206–208°), and TLC ( $R_f$  0.57). The presence of urea (VI) as the methanol-soluble fraction was confirmed by IR and TLC and was comparable with authentic urea ( $R_f$  0.16 and 0.43). The schemes thus indicate the formation path of the degradation products as evidenced by the experimental data.

**Kinetics**—The hydrolytic degradation of I in solution at pH 7.0 and 7.5 at 22.5° and at pH 7.0 at 37° has been shown to be a pseudo-first-order reaction with respect to substrate. A linear relationship was obtained when the log concentration of I was plotted against time. Apparent first-order rate constants were calculated from the slope by least-squares regression, with a correlation coefficient of 0.99. Table I shows apparent rate constants and energy of activation, entropy of activation, and half-life values of I under the conditions studied. Increasing the temperature from 22.5 to 37° accelerated the degradation rate of I 4.5-fold, as expected.

Limited experimental data also were obtained on I stability in water (pH 5.86) and in solutions at pH 1.5 and 4 at 22.5°. The compound was stable up to 76, 39, and 17 days, respectively. A methanolic solution of I ( $9.48 \times 10^{-4} M$ ) showed degradation of 15.10 and 49.82% in 2 and 10 days, respectively.

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# Polynitro Aromatic Compounds in Analytical Chemistry II: Reaction of Menadione with 2,4-Dinitrophenylhydrazine

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**Abstract** □ The intense blue color formed in the official assay of menadione injection by treatment of the sample with 2,4-dinitrophenylhydrazine and ammonia is shown by data from visible light spectra and mass spectra and by comparison of the pKa value with that of the corresponding reaction product of acetone to be due to proton abstraction from a monohydrazone.

**Keyphrases** □ Menadione—blue-colored product of reaction with 2,4-dinitrophenylhydrazine identified □ 2,4-Dinitrophenylhydrazine—blue-colored product of reaction with menadione identified □ Spectrophotometry—analysis, menadione, blue-colored product of reaction with 2,4-dinitrophenylhydrazine identified □ Vitamins—menadione, blue-colored product of reaction with 2,4-dinitrophenylhydrazine identified

The NF (1) assay for menadione (I) consists of heating menadione in alcohol-ether with an acidic solution of 2,4-dinitrophenylhydrazine to give an orange precipitate, followed by the addition of alcoholic ammonia to produce an intense blue color. The absorbance in the visible at 635 nm is proportional to the menadione concentration. The orange precipitate has been assigned Structure II, in which hydrazone formation has taken place at the less hindered

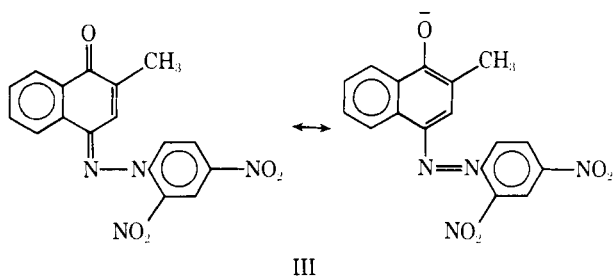
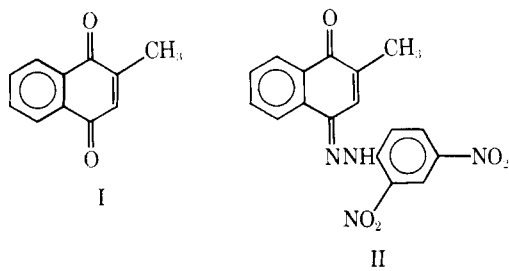
carbonyl (2), but no definitive information seems to be available about the blue material.

In the present work, the composition of the precipitate II was confirmed by mass spectrometry. Since menadione contains a methyl group capable of losing a proton to form an anion, it is possible that Meisenheimer complex formation with the nitro aromatic ring might be responsible for the blue color formation. However, spectrophotometric comparison of the pKa value of menadione 2,4-dinitrophenylhydrazone with that of a simple model compound, acetone 2,4-dinitrophenylhydrazone, suggested that N-deprotonation rather than Meisenheimer complexation was taking place.

## EXPERIMENTAL<sup>1</sup>

**Menadione 2,4-Dinitrophenylhydrazone (II)**—This compound was obtained as orange crystals according to the NF procedure (1). The ma-

<sup>1</sup> Mass spectral data were obtained on a Hitachi Perkin-Elmer RMU 6E instrument. UV-visible spectra were run on a Cary model 15 spectrophotometer. Measurements of pH were made with an ionalyzer, Orion Research model 801 digital pH meter, using a glass-silver-silver chloride combination electrode.



terial was collected by centrifugation and washed with ethanol. The mass spectrum (electron-impact ionization) showed the expected molecular ion ( $M^+$ ) at  $m/e$  352. The isotope peaks at 353 and 354 had intensities of 21 and 4% of  $M^+$ , respectively ( $C_{17}H_{12}N_4O_5$  requires  $M + 1 = 20.1\%$  and  $M + 2 = 2.7\%$ ).

**Acetone 2,4-Dinitrophenylhydrazone**—This compound was prepared as orange crystals according to the method of Brady (3) and was recrystallized from ethanol.

**Determination of Apparent pKa Values for Dinitrophenylhydrazones**<sup>2</sup>—Portions of 2 mg of the dinitrophenylhydrazones were dissolved in 50.0 ml of dimethyl sulfoxide. To 1-ml aliquots of these solutions were added 3 ml of dimethyl sulfoxide, varying amounts of hydrochloric acid or potassium hydroxide, and enough water to bring the volume to 10.00 ml. The apparent pH of each solution was measured using a standardized pH meter. The UV-visible absorption spectra were run between 250 and 650 nm using an appropriate blank.

The positions and intensities of absorption maxima under strongly acid and strongly alkaline conditions were: menadione 2,4-dinitrophenylhydrazone,  $\lambda_{max}$  405 nm ( $\epsilon$  28,400) (acid) and 630 nm ( $\epsilon$  86,800) (alkali); and acetone 2,4-dinitrophenylhydrazone,  $\lambda_{max}$  370 nm ( $\epsilon$  22,700) (acid) and 430 ( $\epsilon$  22,200) and 530 ( $\epsilon$  14,200) nm (alkali).

The families of curves at a range of pH values for both compounds showed an isobestic point, consistent with the presence of only two absorbing species.

The pKa values were calculated from the proportions of the two forms present at an intermediate pH (4, 5). For either compound, the spectrum

at high pH showed a long wavelength maximum, which could be used to estimate the anionic form without interference from the neutral species. Therefore, it was possible to use a single absorbance measurement to determine the fraction of anionic form present at any pH.

**Menadione 2,4-Dinitrophenylhydrazone**—At pH 10.76, the mole fraction of the anionic form was 0.31; therefore, the pKa was 11.10. Measurement was at 630 nm.

**Acetone 2,4-Dinitrophenylhydrazone**—At pH 11.28, the mole fraction of the anionic form was 0.33; therefore, the pKa was 11.58. Measurement was at 530 nm.

## DISCUSSION

The mass spectrometric molecular weight and the intensities of the isotope peaks support the view that the orange precipitate formed initially in the menadione assay has Structure II; it seems unlikely that reaction would take place at the other, more hindered carbonyl group to give the isomeric hydrazone.

A possibility initially entertained was that the color obtained on basifying II was due to formation of a Meisenheimer complex. The nearly instantaneous production of the color argued against this interpretation, which would require dissociation of II to menadione and 2,4-dinitrophenylhydrazone followed by recombination to give the Meisenheimer complex. This process was expected to be relatively slow.

Acetone 2,4-dinitrophenylhydrazone was chosen as a model compound because the modest change in absorbance it shows on basification is not consistent with Meisenheimer complex formation but only with simple *N*-deprotonation. The finding that II has an apparent pKa value similar to that of the acetone derivative suggests that it too is undergoing *N*-deprotonation. The somewhat greater acidity of II is attributable to the more extensive charge delocalization in the anion; two mesomeric representations are illustrated in Structure III. The very intense long wavelength absorption of III is appropriate for a chromophore conjugated over both the dinitrophenyl and the menadione moieties.

In summary, the evidence supports the view that the mesomeric structure of the type shown by III represents the blue-colored species used in the menadione assay.

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<sup>2</sup> All pH and pKa values are apparent.