# The Course of Photooxidation of Menadione*

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Aerobic photolysis of menadione (vitamin $K_3$) in ethanol solution yields menadione 2,3-epoxide which has been analyzed by gas chromatography and characterized by gas chromatography-mass spectometry.

Vitamin K is modified by sunlight (1,2). Aerobic photolysis of phylloquinone (vitamin $K_1$) in benzene solution yields phthiocol (3), and anaerobic photolysis converts phylloquinone in ethanol solution into chromenol (4). Most recently (5), the photolysis of phylloquinone and menaquinones (vitamin $K_2$ group) in cyclohexane solution under aerobic conditions has been investigated and the photooxygenation of naphthoquinones with unsaturated side chains leads to side-chain hydroperoxides. These light-induced structural modifications are interesting and have biological significance in determining its role in oxidative phosphorylation and antihemorrhagic activity.

In view of the biological availability of vitamin K and the ultimate analysis of various K vitamins (6) in animal tissue and blood, we have examined the gas chromatograms and/or mass spectra of menadione upon photolysis. We now report on the effect of sunlight, in presence of air, on menadione in ethanol solution via a photooxygenation reaction.

Authentic menadione is dissolved in 95% ethanol to make up a final concentration of 0.1%. An aliquot of the solution (5 - 30 μg) is injected into a Traco MicroTek MT-220 gas chromatograph equipped with a dual-flame ionization detector.

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Separation of menadione and the oxide is achieved on a 4' x 1/4" I.D. U-glass column packed with 5%(w/w) Silar-5 CP (Applied Science Laboratories, Inc.) on 80-100 mesh Gas Chrom Q at a column temperature 180°C and an inlet temperature of 220°C. The helium carrier gas flow is 80 ml/min. A linear calibration curve between menadione concentration and detector response is established for the standard menadione.

Fig. 1 gives a typical GC analysis of menadione and oxide. The results show that over 50% of the menadione component (peak A) is transformed to the oxide (peak B) and another fraction (peak C) after one minute of sunlight (noon) exposure in the presence of air (Table 1). The retention times of peak A, B, and C are 4, 5.8 and 8 minutes, respectively. Samples of menadione under a nitrogen atmosphere failed to show peaks B and C even after prolonged exposure to sunlight. These observations suggest that photo-induced oxidation occurs only when exogenous oxygen is available.

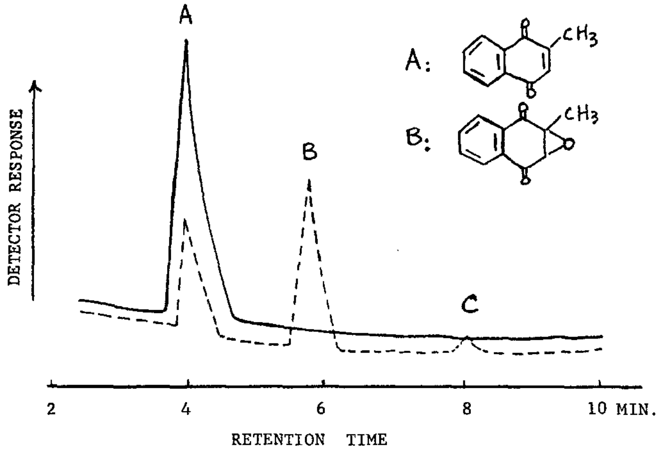

Fig. 1. Gas chromatographic patterns of menadione (Vitamin $K_3$) and oxide derivative obtained from a 4' x 1/2" I.D. U-glass column packed with 5% Silar-5 CP on 80-100 Gas Chrom Q. The inlet temp. 220 C., column temp. 180 C. Helium flow 80 ml/min. Sunlight exposure one min. at noon.

Table 1. Relative retention of menadione in ethanol upon aerobic photolysis

| Exposure time (MIN., noon) | % Menadione (Peak A area) |
|---|---|
| 0 | 100 |
| 1 | 49 |
| 2 | 38 |
| 3 | 29 |
| 4 | 15 |
| 5 | 7 |
| 6 | 0 |

The evidence of mass spectrometer* solid probe and GC-MS analysis of menadione crystal and ethanol mixture with or without aerobic photolysis is presented in Fig. 2. The results show that the pattern of menadione ion fermentation of both solid probe and mixture (GS/MS) are identical, molecular ion = 172, except that the photooxidized menadione sample (GC/MS) shows a different ion fragmentation pattern with molecular ion of 188 - an addition of one oxygen atom. We also note that the characteristic fragmentations between m/e = 172 and m/e = 188 show several molecular ion different patterns. The base peak of menadione is fragment 172 (molecular ion) and that of menadione oxide is 89 (M-99).

Since phylloquinone (vitamin $K_1$) can be converted into its corresponding oxide by treatment of vitamin $K_1$ with hydrogen peroxide (7), we have prepared menadione oxide with the same approach chemically followed by GC analysis and GC/MS identification. The results of the experiments indicate that the chemically oxidized menadione elutes at the same retention time as that of photolytically oxidized menadione on a same GC column. The characteristic MS fragmentation patterns of both chemical and photolytical oxides are in excellent agreement (Fig. 2). An additional test includes the addition of 0.1 N HCl to

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*A Varian Aerograph 1400 GC interfaced with a Finnigan 3000 quadrupole mass spectrometer.

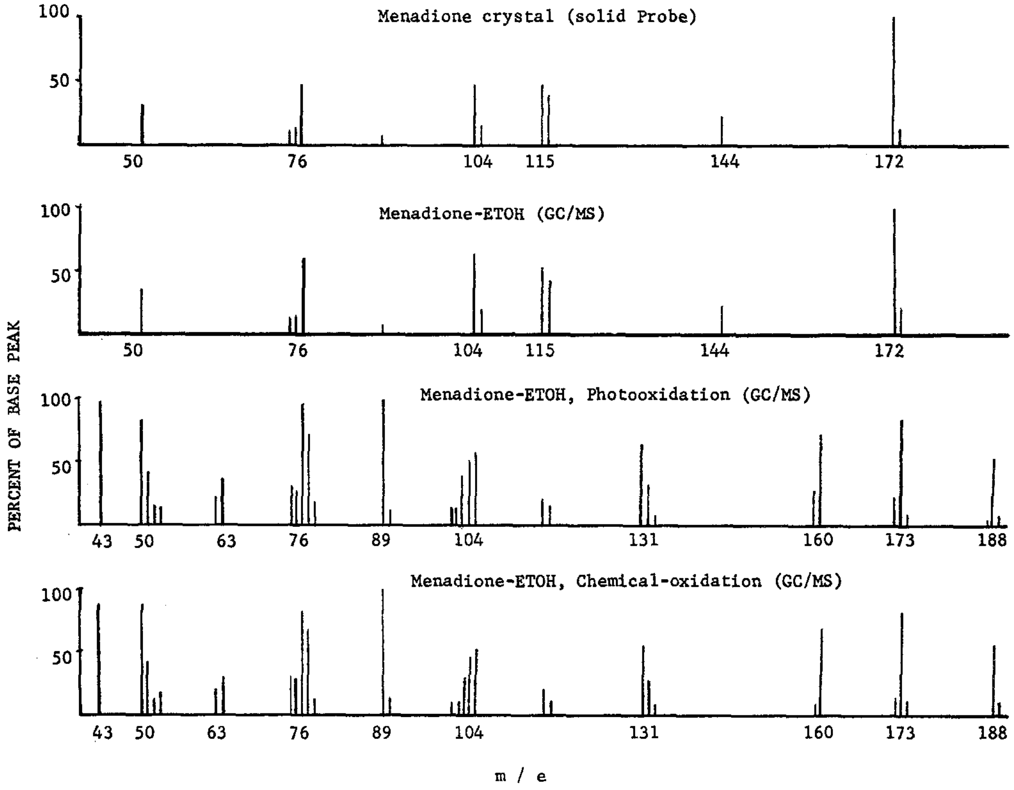

Fig.2. Partial mass spectra of menadiones and menadione oxides. Electron multiplier voltage, 2.0 kv; ionization potential, 70 ev. Solid probe temp., ambient to 125 C. GC column temp., 140 C.

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both oxide mixtures. GC analysis shows that the oxide peak of both acidified mixtures disappears; lability to acids is a feature common to many epoxides. Therefore, it appears that the menadione epoxide is formed by photochemical oxygenation of menadione as well as by chemical epoxidation. Unfortunately, an unambiguous identification of the GC peak C in a mass spectrum is difficult. At present, we are trying to improve the GC system and to extend the method to the analysis of vitamins K in animal.

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