Journal of Chromatography, 110 (1975) 178–181

CHROM. 8240

Note

Gas chromatographic–mass spectrometric investigation of the photo-epoxidation of vitamin K_3*

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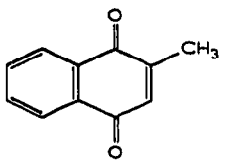
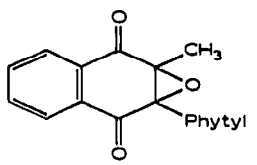
(Received January 28th, 1975)

Vitamin K is widely distributed in plants, animal tissues and in the intestinal tract of most animals and man. However, the vitamin K content as well as its active location in the animal body is not fully understood[1]. Vitamin K has been reported to be affected by sunlight[2], irradiation[3], or radioactivity[4]. But it is also not clear what the significance of these effects on the vitamin K structure might be.

Structurally, vitamin K refers to a group of related compounds which are derivatives of 2-methylnaphthoquinone (I), or vitamin K_3, a synthetic vitamin available in pharmaceutic preparations. Vitamin K_1 oxide (II) has been made chemically by H_2O_2 oxidation, under alkaline conditions, of the parent vitamin also for pharmaceutical purposes[5–7]. Recently, vitamin K_1 oxide has been found as a metabolite of the parent vitamin in a system which forms the 2,3-epoxide and regenerates the vitamin in a cyclic fashion[8,9], the metabolism playing an important role in the physiological regulations of prothrombin synthesis[10].



I



II

Gas chromatography (GC) and mass spectrometry (MS) have been found to be useful for analysis[11–14] and characterization[15] of vitamin K. In the course of studies on the availability of vitamin K by the GC technique[16], it was found that a second GC peak appears at the longer retention time which is distinct from the vitamin K_3 peak during the routine calibration check of the standard solution stored under normal indoor lighting conditions. The studies here indicate that vitamin K_3 is readily converted to vitamin K_3 oxide with sunlight in the presence of oxygen, and suggest that the photo-epoxidation reaction involves a singlet molecule oxygen receiving electrons

* Journal Series No. 1843 of the Hawaii Agricultural Experiment Station.

from the vitamin K_3 molecule in a simple system containing vitamin K_3, ethanol and exogenous oxygen.

EXPERIMENTAL

Pure menadione, vitamin K_3 (N.B.C., Cleveland, Ohio, U.S.A.) was dissolved in 95% ethanol to make up a final concentration of 0.1%. The gas chromatograph used in this study was an MT-220 (Tracor, Austin, Texas, U.S.A.), with a four-column oven, and equipped with a dual-flame ionization detector. A 6 ft. $\times$ $\frac{1}{4}$ in. I.D. U-glass column packed with 5% (w/w) Silar-5CP (Applied Science Labs., State College, Pa., U.S.A.) on 80–100 mesh Gas-Chrom Q was used for the analysis of vitamin K and its derivative. The column temperature was 180° and the inlet temperature 220°. The carrier gas (helium) flow-rate was 50 ml/min. A linear calibration curve between the vitamin K_3 (menadione) concentration and the detector response was established for the standard vitamin (5–30 μg).

Samples of menadione crystal and standard ethanolic menadione were used for mass spectrometry. The instruments used were a Varian-Aerograph 1400 gas chromatograph interfaced via a Finnigan 3000 quadrupole mass spectrometer. The probe containing the crystal was placed into the ionization chamber at ambient temperature and 70 eV and the electron multiplier voltage was 2.0 kV. The ethanolic vitamin mixture was injected into a 3 ft. $\times$ 0.079 in. I.D. glass column of the same packing material as that stated above. The column temperature was 140° and the inlet temperature 210°. The helium flow-rate was 25 ml/min.

Chemical oxidation was carried out by reacting 1 ml of menadione standard (0.01% in ethanol) with 0.1 ml of H_2O_2 (30%) and 0.1 ml of Na_2CO_3 (10%). Photo-oxidation was conducted under the sunlight exposure at noon with or without the enclosure of air, oxygen or nitrogen.

RESULTS AND DISCUSSION

Fig. 1 shows the GC curves of the separation and the patterns of menadione, menadione oxide and the derivative of the vitamin under the various experimental conditions. The results show that over 50% of the menadione (peak a) is converted into the oxide (peak b) and the derivative (peak c) after sunlight reaction (1 min, noon) in the presence of air (Fig. 1B). The retention times of peaks a, b, and c are 4, 5.8 and 8 min, respectively. Samples of menadione mixture under the nitrogen atmosphere failed to show peaks b and c even under the prolonged exposure to sun-light (Fig. 1c). These observations suggest the possibility that in this case the photo-induced oxidation only occurred when exogenous oxygen was available. For example, 95% or more of the menadione (0.01% in ethanol) was converted into the oxide form when exposed to sunlight at noon for 10 min in the presence of oxygen (Fig. 1C). The chemical conversion of vitamin K_3 to vitamin K_3 oxide was achieved with alkaline H_2O_2. The chemically induced oxides elute at the same retention time as do the photo-induced oxides on a the GC column under the same operation conditions. The effect of acid on the oxide resulting from both sunlight- and H_2O_2-treated samples is shown in Fig. 1D. The poor recovery of the peak c component upon the disappearance of peak b demonstrates the destructive effect of acid on the oxide and the increase in

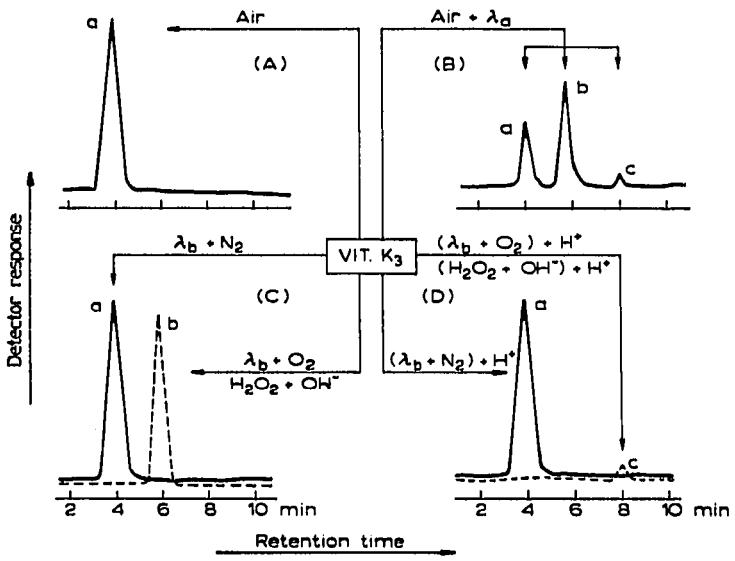


Fig. 1. GC curves showing the elution, the retention, and the patterns of menadione (a), menadione oxide (b) and derivative (c) as influenced by sunlight in the presence of air, oxygen, nitrogen, and acid. 5% (w/w) Silar-5CP on 80–100 mesh Gas-Chrom Q, 6 ft. × ¼ in. I.D. U-glass. Column temperature, 180°, inlet temperature, 220°. Helium flow-rate, 50 ml/min. λ_a = 1 min and λ_b = 10 min sunlight at noon.

polarity of the degraded product(s). Application of light to vitamin K_3 or oxygen alone and immediate recombination of the two reactants failed to show any sign of photo-induced epoxidation of the vitamin. It is suggested, therefore, that the photo-epoxidation involves a singlet molecule oxygen (excited state) receiving electrons from the vitamin K_3 molecule to form a peroxy anion (O_2^{2-}). The peroxy anion has been known to have the O–O bond lengthened to such an extent that the oxygen molecule splits into two atoms, and photo-epoxidation takes one single oxygen to complete.

The mass spectra of vitamin K_3 and its oxide regarding fragmentation of the molecule are shown in Table I. The relative abundance of the ten most prominent ions in the mass spectra indicates that the molecular ion of vitamin K_3 is 172 for both crystal (solid probe) and mixture (GC–MS), and that that of vitamin K_3 oxide is 188 for both photo-induced and chemically induced products. Addition of one oxygen atom to the vitamin K_3 molecule is therefore characterized by GC–MS. It is noted that the characteristic fragmentations between *m/e* 172 and *m/e* 188 show several different patterns. The masses of vitamin K_3 (in solution) and that of crystal (solid probe) and those of their subsequent fragments are identical, suggesting the high stability of vitamin K_3 solution during GC analysis. On the other hand, the characteristic fragmentation pattern of the photo-induced oxide corresponds essentially to that of the chemically induced oxide. Thus, there is a structural similarity between the photo-oxygenated menadione and the chemically oxygenated menadione —both combine with one oxygen atom to yield an oxide or epoxide. The application of these studies should be useful in attempts to unravel the intricacies of the physiological actions of vitamin K.

TABLE I

RELATIVE ABUNDANCE OF TEN MOST PROMINENT IONS IN MASS SPECTRA OF VITAMIN K_3 AND ITS OXIDE

Vitamin K_3				*Vitamin K_3 oxide*			
Solid probe (crystal)		*GC–MS (N_2-λ)*		*GC–MS (O_2-λ)*		*GC–MS (H_2O_2)*	
m/e	*RA**	*m/e*	*RA**	*m/e*	*RA**	*m/e*	*RA**
172**	100	172**	100	188**	52	188**	55
144	24	144	22	173	86	173	81
116	40	116	43	160	69	160	68
115	46	115	51	131	60	131	52
105	16	105	20	105	62	105	52
104	47	104	62	104	52	104	45
76	49	76	59	89	100	89	100
74	13	74	13	76	95	76	82
50	31	50	35	50	81	50	87
39	21	—	—	43	95	43	87

* Relative abundance with *m/e* in each case.
** Molecular ion.

ACKNOWLEDGEMENTS

The authors thank Dr. D. M. Hilker, Department of Food and Nutritional Sciences, University of Hawaii, for her assistance in discussion and reviewing this manuscript. This work was supported in part by a grant from the Research Council, University of Hawaii, Hawaii, U.S.A.

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