ACTA CHEMICA SCANDINAVICA 24 (1970) 2839–2844

Photoinduced Fluorescence of Phylloquinone (Vitamin K_1) in Solution

OWE JANSSON

Institute of Botany, University of Stockholm, Lilla Frescati, S-104 05 Stockholm 50, Sweden

Phylloquinone that is not normally fluorescent in petroleum ether or ethanol solutions at room temperature was found to exhibit a photoinduced fluorescence when the solutions were irradiated with the 365 nm mercury line. With prolonged irradiation, the fluorescence intensity increased gradually to a maximum and then decreased. Exclusion of oxygen from the solutions had no observable effect on their photoinduced fluorescence. Thin-layer chromatograms of the irradiated solutions indicated that the fluorescence arises from an intermediate in the photolysis of phylloquinone. The intensity of the fluorescence produced in petroleum ether was found to be directly proportional to the concentration of phylloquinone in the range of 5–20 μg/ml.

Fluorescence was not obtained when petroleum ether solutions of menadione, plastoquinone-9, or ubiquinone-10 were irradiated in the same manner as phylloquinone.

Phylloquinone (2-methyl-3-phytyl-1,4-naphthoquinone) exhibits a green fluorescence on filter paper and on thin layers of silica gel G after prolonged irradiation with ultraviolet light.[1–4] Green and Dam [2] have shown that the green fluorescence on filter paper is due to a photochemical reaction product of phylloquinone.

Green [1] was unable to detect fluorescence of phylloquinone in petroleum ether or ethanol solutions. Phylloquinol does not fluoresce in aqueous solutions, although 2-methyl-1,4-naphthohydroquinone shows a strong fluorescence.[5,6]

If 2-hydroxy-3-dihydroprenyl-1,4-naphthoquinone in ethereal solution is exposed to ultraviolet light, it is gradually destroyed with the appearance of a blue fluorescence.[7] Hercules and Surash [8] found that 1,4-naphthoquinone, although not normally fluorescent in ethanolic solution at room temperature, exhibits a violet fluorescence after prolonged irradiation with ultraviolet light when oxygen has been excluded from the solution. They concluded that the photoinduced fluorescence arises from intermediates in the photoreduction of 1,4-naphthoquinone.

Katsui and Ohmae [9-13] have studied the photolysis of phylloquinone in benzene or alcohol solutions during ultraviolet irradiation. Many coloured or fluorescent decomposition products were detected on thin-layer chromatograms of the irradiated solutions. Some of the products were identified.

The present paper describes some characteristics of a photoinduced fluorescence of phylloquinone in petroleum ether or ethanol solutions. The origin of the fluorescence has been investigated by means of thin-layer chromatography. In addition, menadione (2-methyl-1,4-naphthoquinone), plastoquinone-9 (2,3-dimethyl-6-nonaprenyl-1,4-benzoquinone), and ubiquinone-10 (2,3-dimethoxy-5-methyl-6-decaprenyl-1,4-benzoquinone) have been tested for fluorescence emission under the same experimental conditions as phylloquinone.

EXPERIMENTAL

Chemicals. Phylloquinone was obtained from Nutritional Biochemicals Corporation, Cleveland, Ohio, USA, and ubiquinone-10 from Sigma Chemical Company, St. Louis, Missouri, USA. Plastoquinone-9 was supplied by Dr. O. Isler, F. Hoffmann-La Roche & Co., Basle, Switzerland. Menadione was obtained from E. Merck AG, Darmstadt, Germany.

Spectrographically pure ethanol was purchased from AB Vin- & Spritcentralen, Stockholm, Sweden. Analytical grade petroleum ether having a boiling range of 30—60°C was obtained from Mallinckrodt, New York, N.Y., USA. Benzene, chloroform, sulphuric acid, and rhodamine B were analytical grade products of E. Merck AG. Silica gel G according to Stahl for thin-layer chromatography was also from E. Merck AG.

All chemicals were used without further purification.

Fluorescence measurements were made with a Zeiss spectrophotometer PMQ II connected with its fluorescence attachment ZFM 4. The exciting radiation (365 nm) was isolated from an St 41 mercury lamp by a filter. Lidded glass cuvettes of 10 mm light path were used as containers for samples (3.2 ml) of the solutions to be assayed. The emitted fluorescence was passed through the monochromator of the spectrophotometer for dispersion. The monochromator slit was always set at 0.4 mm. An RCA 1P28 photomultiplier tube was used as fluorescence detector. The output of the detector was amplified to a high degree. Right angle arrangement was utilized for placing the cuvette with respect to the excitation and detection components. The measurements were carried out at 22°C in a dark room illuminated by a dim electric light.

The fluorescence spectra were not corrected for the spectral response of the photomultiplier tube, for the variation of the band width during scanning with constant slit width, and for the loss of light within the optical equipment between sample and detector.

Petroleum ether or ethanol were used as solvents. They showed a minute fluorescence at the level of sensitivity used for measurement. Corrections were always introduced for the background emission of the solvent blanks.

Irradiation of solutions. Samples of solutions were irradiated directly in the cuvette compartment of the fluorescence attachment with the 365 nm mercury line. The same radiation served to cause photolysis and to excite fluorescence.

Thin-layer chromatography. Glass plates (20 × 20 cm) were coated with a layer of silica gel G, 0.25 mm thick. After an activation period of 30 min at 100—110°C, the plates were stored in desiccators until just prior to use. Aliquots (0.1 ml) of the solutions to be chromatographed were applied to starting points 1.5 cm from the lower end of the plate. The chromatogram was developed with benzene-chloroform (1:1 v/v) in darkness at 25°C. About 30 min was sufficient for the solvent front to ascend 15 cm from the starting points. At this time the plate was removed from the chromatography jar and then dried for a few minutes at room temperature.

Three procedures were employed for the detection of substances on the developed chromatograms. (1) Some plates were exposed to ultraviolet light (253.7 or 365.0 nm) from a UA-220 lamp (Lumalampan AB, Stockholm, Sweden) for 20 min. Fluorescent spots appeared on the plates. (2) Other chromatograms were sprayed with a solution

of 0.25 % (w/v) rhodamine B in ethanol and then examined under the ultraviolet lamp. The visualized substances showed a violet fluorescence on an orange-coloured background. (3) Some plates were heated for 10 min at 130°C and then sprayed with concentrated sulphuric acid. Coloured spots appeared on the chromatograms.

RESULTS AND DISCUSSION

Relationship between irradiation time and fluorescence intensity. Fig. 1 shows the appearance of a photoinduced fluorescence at room temperature when solutions of phylloquinone in petroleum ether or in ethanol were exposed to prolonged irradiation with the 365 nm mercury line.

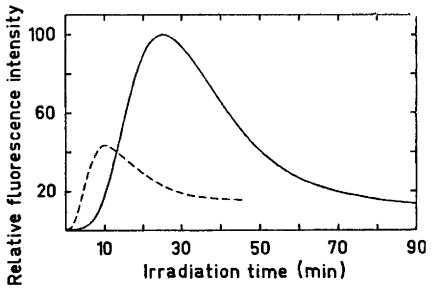

Fig. 1. Variation of the photoinduced fluorescence intensity of phylloquinone with time of irradiation. Concentration: 200 μg/ml. The fluorescence intensity was measured at the fluorescence maximum (ethanol solution, 505 nm; petroleum ether solution, 431 nm) at intervals of half a minute. ———, fluorescence produced in ethanol; — — —, fluorescence produced in petroleum ether.

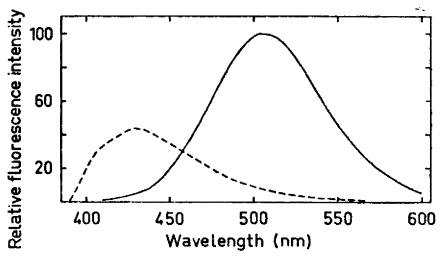

Fig. 2. Photoinduced fluorescence spectra of phylloquinone in ethanol, ———, and in petroleum ether, — — —. Concentration: 200 μg/ml. The spectra were measured rapidly at intervals of 5 nm when the solutions exhibited their strongest fluorescence (see Fig. 1). Corrections were not introduced for the response characteristics of the detector system.

In some experiments the same solutions were bubbled with nitrogen for 15 min immediately before irradiation in order to exclude oxygen. The deoxygenation had no observable effect on the fluorescence emission.

The photoinduced fluorescence spectra of phylloquinone in petroleum ether or ethanol solutions are shown in Fig. 2.

Effect of ultraviolet irradiation. Samples of phylloquinone in petroleum ether (200 μg/ml) were exposed to the 365 nm mercury line for different periods corresponding to different stages of the photoinduced fluorescence emission (*cf.* Fig. 1). The solution was not deoxygenated prior to irradiation. After that the samples were compensated for evaporated solvent and then analysed by means of thin-layer chromatography. Fig. 3 shows a typical chromatogram which is based upon several developed thin-layer plates.

Samples of phylloquinone in ethanol (200 μg/ml) were irradiated and analysed in the same manner. A typical chromatogram is shown in Fig. 4.

The substances on the chromatograms were visualized by various procedures. The results are summarized in Table 1.

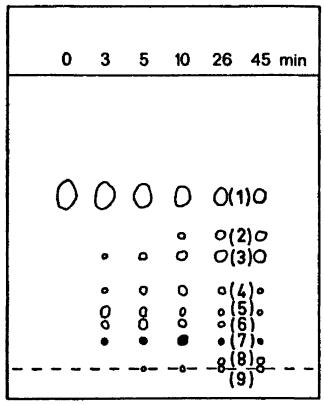

Fig. 3. Thin-layer chromatogram of products formed by ultraviolet irradiation of phylloquinone in petroleum ether (200 μg/ml). Irradiation time was noted at the top of the silica gel G plate. Development with benzene-chloroform (1:1 v/v).

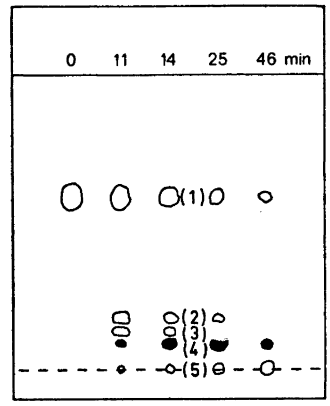

Fig. 4. Thin-layer chromatogram of products formed by ultraviolet irradiation of phylloquinone in ethanol (200 μg/ml). Irradiation time was noted at the top of the silica gel G plate. Development with benzene-chloroform (1:1 v/v).

Table 1. R_F values of the substances in Figs. 3 and 4 and the visualization of these substances on the chromatograms by means of various detection procedures (see Experimental). +=spots appeared; −=no spots appeared.

No. of substance	R_F	Detection				
		Visible light	Ultraviolet light [a]		Rhodamine B [a]	H_2SO_4
			Instantly	After prolonged exposure		
Fig. 3: 1	0.59	+	−	+	+	+
2	0.45	−	−	+	+	−
3	0.38	−	−	+	+	+
4	0.27	−	−	+	+	−
5	0.19	−	−	+	+	+
6	0.15	−	−	+	+	+
7	0.09	−	+	+	−	+
8	0.02	+	−	−	+	−
9	0.00	+	−	−	+	+
Fig. 4: 1	0.59	+	−	+	+	+
2	0.18	−	−	+	+	+
3	0.13	−	−	+	+	+
4	0.09	−	+	+	+	+
5	0.00	+	−	−	+	+

[a] The same result was obtained in short-wave ultraviolet (253.7 nm) as in long-wave ultraviolet light (365.0 nm).

The same chromatographic patterns were obtained when the irradiated solutions were kept in darkness at 22°C for 18 h before analysis.

No spots could be detected on developed plates when the solvents were irradiated with the 365 nm mercury line and then subjected to thin-layer chromatography.

It is evident from Figs. 3 and 4 that phylloquinone (spot 1) was gradually destroyed on exposure to the ultraviolet light with the formation of many decomposition products. Some products (spots 4–7 in Fig. 3; spots 2–4 in Fig. 4) appeared in the initial stage of the irradiation and then decreased in amount; they are intermediates in the photolysis of phylloquinone. Other products (spots 2, 3, 8, 9 in Fig. 3; spot 5 in Fig. 4) increased in the later stage of the irradiation; they are final decomposition products. The mechanism of the photolysis in petroleum ether seems to differ from the mechanism of the decomposition in ethanol.

The photoinduced fluorescence of phylloquinone in petroleum ether or ethanol solutions arises probably for the most part from an intermediate in the photolysis of phylloquinone. In support of this assumption the following can be mentioned: (1) An intermediate (spot 7 in Fig. 3; spot 4 in Fig. 4) was the only substance that fluoresced immediately when chromatograms of the irradiated solutions were exposed to ultraviolet light (Table 1). All other fluorescent spots appeared only after prolonged irradiation. (2) The variation in size of the immediately fluorescing spots on each thin-layer plate corresponded to the variation in final fluorescence intensity among the irradiated samples of phylloquinone in solution (*cf.* Figs. 1, 3, and 4).

Relationship between concentration and fluorescence intensity. Freshly prepared solutions of phylloquinone in petroleum ether or in ethanol were exposed to prolonged ultraviolet irradiation. The intensity of the photoinduced fluorescence was measured during the irradiation. Figs. 5 and 6 show plots of the highest fluorescence intensity against the logarithm of the concentration of

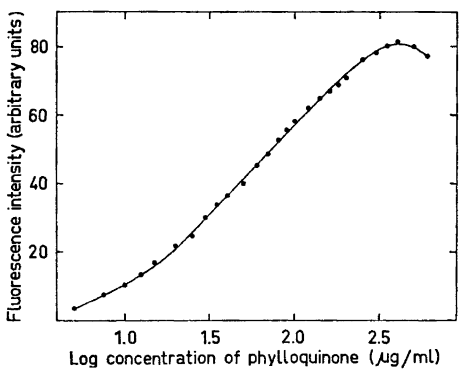

Fig. 5. Effect of concentration on the photoinduced fluorescence intensity of phylloquinone in petroleum ether. The highest fluorescence intensity at 431 nm was plotted.

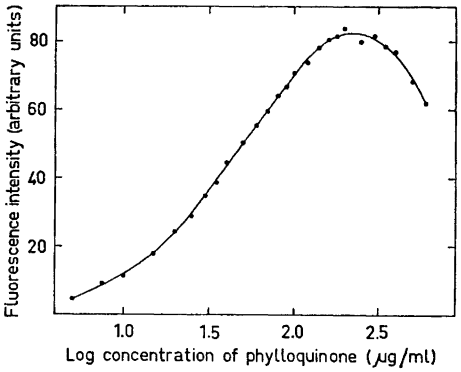

Fig. 6. Effect of concentration on the photoinduced fluorescence intensity of phylloquinone in ethanol. The highest fluorescence intensity at 500 nm was plotted.

phylloquinone. All petroleum ether solutions had a fluorescence maximum at 431 nm (uncorrected). The highest intensity of the fluorescence produced in petroleum ether was directly proportional to the concentration of phylloquinone in the range of 5 – 20 μg/ml. At greater concentrations, the emission intensity went through a maximum and then decreased. That was probably due to the presence of an inner filter effect. When ethanol was used as the solvent, the fluorescence maximum shifted to longer wavelength as the concentration of phylloquinone was raised, *e.g.*: 5 μg/ml, 480 nm; 25 μg/ml, 490 nm; 90 μg/ml, 500 nm; 180 μg/ml, 505 nm; 350 μg/ml, 510 nm (uncorrected instrumental readings). Consequently, the photoinduced fluorescence in ethanol cannot be utilized for quantitative assay of phylloquinone.

Fluorescence testing of other quinones. Solutions of menadione, plastoquinone-9, or ubiquinone-10 in petroleum ether (40 and 200 μg/ml) were exposed to the 365 nm mercury line for 30 min and continuously tested for fluorescence emission. The solutions were not deoxygenated prior to irradiation. No fluorescence was emitted from the solutions.

The failure of menadione to exhibit fluorescence shows that in some way the phytyl group of phylloquinone is essential to the production of photoinduced fluorescence in petroleum ether.

Acknowledgements. The author is indebted to Professor Torsten Hemberg for his interest in this work, to Professor Göran Schill for scrutinizing the manuscript, and to Dr. Otto Isler for the supply of plastoquinone-9. This investigation was financed in part by *Anslaget till främjande av ograduerade forskares vetenskapliga verksamhet.*

REFERENCES

1. Green, J. P. *Nature* **174** (1954) 369.
2. Green, J. P. and Dam, H. *Acta Chem. Scand.* **8** (1954) 1341.
3. Bollig, H. R. In Stahl, E. *Dünnschicht-Chromatographie*, Springer-Verlag, Berlin, Göttingen, Heidelberg 1962, p. 240.
4. Lichtenthaler, H. K. and Sprey, B. *Z. Naturforsch.* **21b** (1966) 690.
5. Duggan, D. E., Bowman, R. L., Brodie, B. B. and Udenfriend, S. *Arch. Biochem. Biophys.* **68** (1957) 1.
6. Udenfriend, S., Duggan, D. E., Vasta, B. M. and Brodie, B. B. *J. Pharmacol. Exptl. Therap.* **120** (1957) 26.
7. Ettlinger, M. G. *J. Am. Chem. Soc.* **72** (1950) 3666.
8. Hercules, D. M. and Surash, J. J. *Spectrochim. Acta* **19** (1963) 788.
9. Katsui, G. and Ohmae, M. *J. Vitaminol. (Osaka)* **12** (1966) 117.
10. Ohmae, M. and Katsui, G. *Vitamins (Kyoto)* **35** (1967) 116.
11. Ohmae, M. and Katsui, G. *Vitamins (Kyoto)* **39** (1969) 181.
12. Ohmae, M. and Katsui, G. *Vitamins (Kyoto)* **39** (1969) 190.
13. Ohmae, M. and Katsui, G. *Vitamins (Kyoto)* **41** (1970) 178.

Received March 4, 1970.