Journal of Chromatography, 196 (1980) 506–511
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 12,904

Note

Preparation, separation and characterization of vitamin E quinone

GUNDU H. R. RAO*
Department of Laboratory Medicine and Pathology, University of Minnesota, Minneapolis, MN 55455 (U.S.A.)
THOMAS P. KRICK
Department of Biochemistry, University of Minnesota, Minneapolis, MN 55455 (U.S.A.)
and
JAMES G. WHITE
Departments of Laboratory Medicine and Pathology, and Pediatrics, University of Minnesota, Minneapolis, MN 55455 (U.S.A.)
(Received April 4th, 1980)

Vitamin E (α-tocopherol) is a required nutrient, first identified by Evans and Bishop[1] in 1922, which has been the focus of new interest since clinical trials suggested it might be effective in reducing the incidence of certain thrombolic disorders, including intermittent claudication[2–6], cerebral arteriosclerosis[7,8] and, possibly, angina and coronary-artery disease[9–13]. However, recent studies in this laboratory have suggested that the oxidation product of vitamin E, vitamin E quinone (α-tocopherol quinone), is a more potent inhibitor of platelet aggreation than the parent compound[14]. As a result, the focus of interest has shifted to metabolites of vitamin E as potential antithrombotic agents.

One difficulty, however, is the preparation of large amounts of pure metabolites of vitamin E for *in vitro* and *in vivo* testing. Procedures necessary to obtain quantities of metabolites, such as α-tocopherol quinone, are complex and require difficult preparative methods of analytical chemistry[15]. The purpose of this report is to describe a simple procedure for obtaining large amounts of pure α-tocopherol quinone that retains its biological activity.

MATERIALS AND METHODS

A Waters Assoc. Model 204 high-performance liquid chromatograph was used for the separation of products. The system consisted of a Model 440 fixed-wavelength (254 nm) UV detector, a 6000A solvent-delivery system and a UK6 universal injector. A 30-cm stainless-steel column, packed with μBondapak C_{18} was used for the separation work, and the mobile phase was pure acetonitrile. A Hewlett-Packard 3385-A automation system was used to obtain electronic integration of the peaks. Authentic samples of α-tocopherol and α-tocopherol quinones were a gift from Dr. Takehito Oki (Elasi Co., Tokyo, Japan). Oxidized products of vitamin E and the parent compound were subjected to electron-impact mass spectrometry (direct-inlet technique) using an

0021-9673/80/0000–0000/$02.25 © 1980 Elsevier Scientific Publishing Company

LKB 9000 mass spectrometer with a digital PDP-8e data processor. For simplicity, we will refer to the oxidation product obtained from vitamin E in our study as vitamin E quinone and to the authentic compound from the commerical source as α-tocopherol quinone.

RESULTS AND DISCUSSION

Preparation of vitamin E quinone

Vitamin E quinone was prepared by oxidation of 100 mg of α-tocopherol with 5–10 drops of concentrated nitric acid at room temperature for 2 or 3 min. The oxidized material was thoroughly rinsed several times with distilled water until the pH of the rinse water was greater than 5; the residue was then dissolved in absolute alcohol.

High-performance liquid chromatographic analysis of the oxidation products

Vitamin E and aliquots of the oxidized vitamin E were subjected to high-performance liquid chromatography (HPLC). Vitamin E gave a single major peak at a retention time of 8.15 min (Fig. 1). Sub-samples from the oxidized vitamin E preparation also gave a single major peak at 6.49 ± 0.02 min. Subsequently, a mixture containing vitamin E and vitamin E quinone were subjected to analysis by liquid chromatography. Good separation of vitamin E from vitamin E quinone was achieved, and the retention times matched those of the individual compounds (Fig. 2).

To further identify the oxidized product of vitamin E, authentic α-tocopherol quinones were run under the same analytical conditions. α-Tocopherol quinone

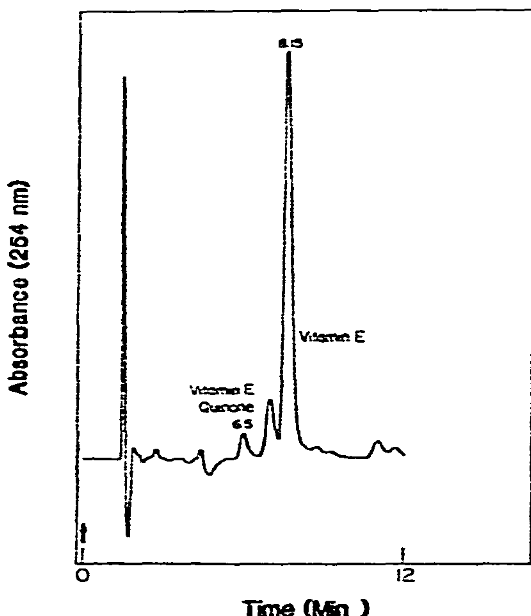

Fig. 1. Chromatogram showing separation of vitamin E by HPLC. Column, 30 cm × 4 mm I.D.; packing, μBondapak C_{18}; solvent, 100% acetonitrile; detector, UV 254 nm; sample, 10 μl.

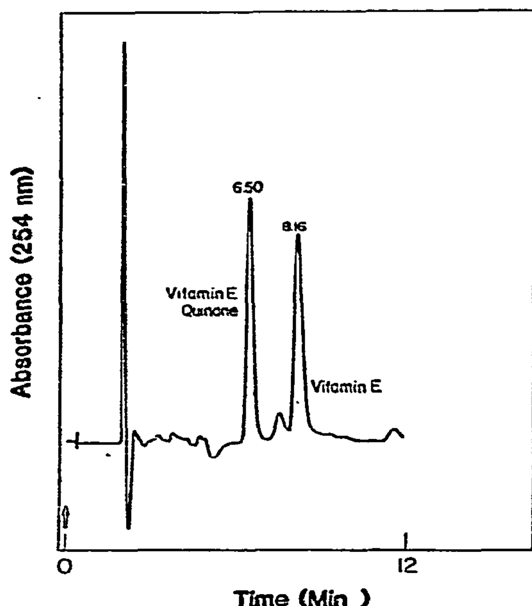

Fig. 2. Chromatogram showing separation of vitamin E and vitamin E quinone. Good separation was achieved and the retention times matched those of the individual compounds as shown in Fig. 1. Conditions as in Fig. 1.

($C_{29}H_{50}O_3$; mol.wt. 446), α-tocopherol quinone model ($C_{14}H_{20}O_3$; mol.wt. 236), α-phytyl quinone ($C_{29}H_{48}O_2$; mol.wt. 428) and vitamin E_2 $C_{29}H_{42}O_2$; mol. wt. 422) showed retention times of 6.47, 3.0, 12.5 and 6.1 min, respectively. Vitamin E quinone and α-tocopherol quinone showed retention times of 6.49 $\pm$ 0.01 min (Fig. 3).

UV spectral studies of vitamin E quinone

Vitamin E, vitamin E quinone and α-tocopherol quinone were scanned for their UV absorption characteristics. Vitamin E had an absorption maximum at 292 nm, whereas vitamin E quinone and α-tocopherol quinone showed maximum absorption at 269 nm.

Characterization of vitamin E quinone

Vitamin E quinone prepared in this laboratory and isolated by HPLC was subjected to mass spectral analysis; an authentic standard of α-tocopherol was also analyzed for comparison. Results on the major mass fragmentation, percentages of maximum single ions and the complete spectra are presented in Table I and Fig. 4. Vitamin E quinone had major ion peaks at 446, 221 and 178 and matched exactly with the fragmentation pattern of authentic α-tocopherol quinone.

Biological activity of vitamin E quinone

Vitamin E quinone prepared by the method described in this paper inhibited platelet aggregation and secretion induced by various aggregating agents. The potency

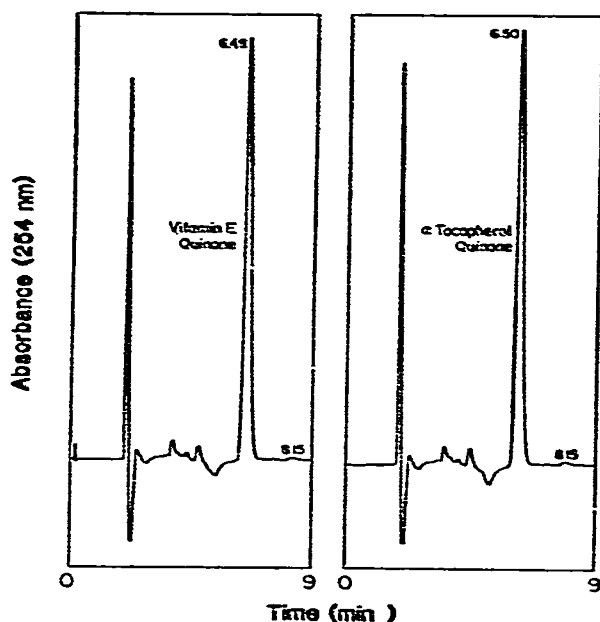

Fig. 3. Chromatogram showing separation of vitamin E quinone and α-tocopherol quinone by HPLC; vitamin E quinone and α-tocopherol quinone showed retention times of 6.49 min.

TABLE I

MASS SPECTRAL ANALYSIS OF VITAMIN E (VE), VITAMIN E QUINONE (VEQ) AND α-TOCOPHEROL QUINONE (ATQ)

Vitamin E and its oxidized product were analyzed by MS, and the results were compared with the data obtained using authentic α-tocopherol. Fragmentation patterns obtained with these compounds agree with the major ion peaks obtained with the oxidized product of vitamin E matched with those of authentic α-tocopherol quinone.

Compound	*Major mass fragments (daltons)*		*Percentage of maximum single ions*	
	This work	*Published**	*VEQ or ATQ*	*VE*
VEQ or ATQ	446	446	10.0	0.2
	221	221	100.0	1.9
	178	178	74.0	1.9
VE	430	430	7.1	100.0
	205	205	4.7	9.8
	165	165	24.9	63.0

* See ref. 16.

of the compound was similar to that of authentic α-tocopherol quinone and was 5–10 times higher than that of vitamin E.

In summary, vitamin E was oxidized in the present study with concentrated nitric acid, and the acid-free extract was subjected to HPLC. The oxidation procedure yielded a single major product, which matched the retention time of authentic α-tocopherol quinone and exhibited similar UV absorption characteristics. Mass

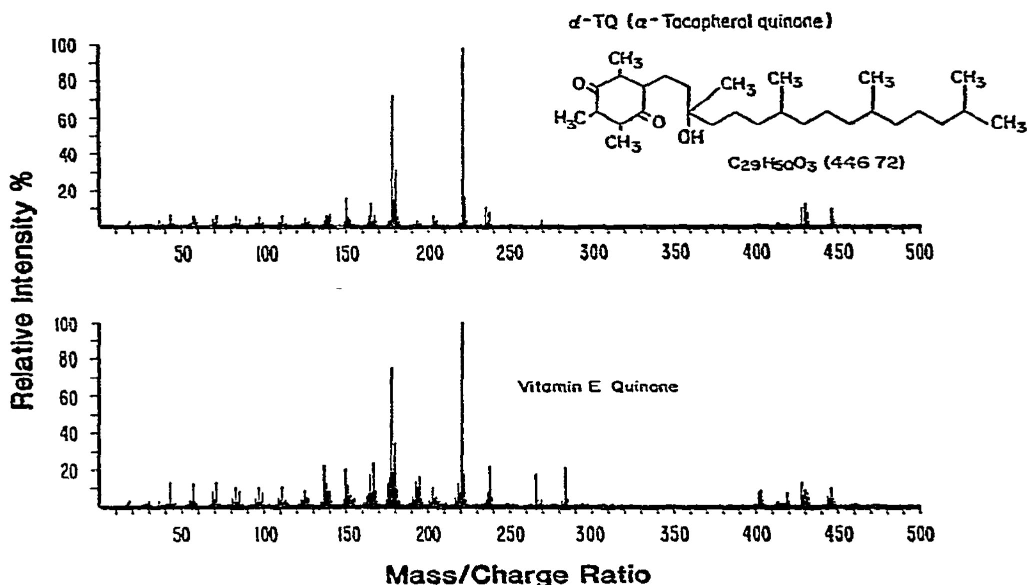

Fig. 4. Mass spectra of vitamin E quinone and authentic α-tocopherol quinone. Fragmentation patterns of the major ion peaks of vitamin E quinone matched those of α-tocopherol quinone.

spectral analysis confirmed the qualitative information obtained by liquid chromatographic analysis and showed that the major product of nitric acid-induced oxidation was α-tocopherol quinone.

Biological activity of the vitamin E quinone prepared by the simple oxidation procedure described here was 5–10 times higher than that of vitamin E in inhibiting platelet function. The method described permits rapid preparation and separation of pure vitamin E quinone for experimental studies.

ACKNOWLEDGEMENTS

This work was supported by USPHS Grants HL-11880, AM-06317, HL-06314, CA-12607, CA-08832, CA-11996, GM-AM-22167, HL-20695, HL-16833 and AM-15317. The authors wish to thank the University of Minnesota Agriculture Experiment Station for the gas chromatograph–mass spectrometer facility.

REFERENCES

1 H. M. Evans and K. S. Bishop, *Science*, 56 (1922) 650.
2 E. V. Shute, A. B. Vogelsang, F. R. Skelton and W. E. Shute, *Surg. Gyn. Obstet.*, 86 (1948) 1.
3 A. M. Ratcliffe, *Lancet*, ii (1949) 1128.
4 P. D. Livingstone and C. Jones, *Lancet*, ii (1958) 602.
5 A. M. Boyd and J. Marks, *Angiology*, 14 (1963) 198.
6 H. T. G. Williams, D. Fenna and R. A. MacBeth; *Surg. Gyn. Obstet.*, 132 (1971) 662.
7 C. Naito, A. Ohta, R. Ohmure, H. Okaniwa, K. Ogawa, K. Goto, H. Komwa, and F. Nakada, *Igaku no Ayumi*, 91 (1974) 628.
8 K. Iino, K. Abe, S. Kariya, H. Kimura, T. Kusaba, R. Kusunoki, J. Saku, K. Soejima, S. Naka-

kura, I. Nakamura, K. Nakamura, Y. Nakayama, A. Notomi, M. Hanada, T. Miyazaki and I. Furukawa, *Jap. Heart J.*, 18 (1977) 277.
9 A. Vogelsang and E. V. Shute, *Nature (London)*, 157 (1946) 772.
10 A. Vogelsang, E. V. Shute and W. E. Shute, *Med. Record*, 160 (1947) 91.
11 S. H. Rinzler, H. Bakst, Z. H. Benjamin, A. L. Bobb and J. Travell, *Circulation*, 1 (1950) 288.
12 W. M. Toone, *New Engl. J. Med.*, 289 (1973) 979.
13 T. W. Anderson, *Gen. Med. Assoc. J.*, 110 (1974) 401.
14 A. C. Cox, G. H. R. Rao, J. M. Gerrard and J. G. White, *Blood*, 55 (1980) 907.
15 J. Green and D. McHale, in R. A. Morton (Editor), *Biochemistry of Quinones*, Academic Press, New York, 1965, p. 261.
16 W. H. Elliot and G. R. Waller, in G. R. Waller (Editor), *Biochemical Applications of Mass Spectrometry*, Wiley-Interscience, New York, 1972, pp. 499–532.