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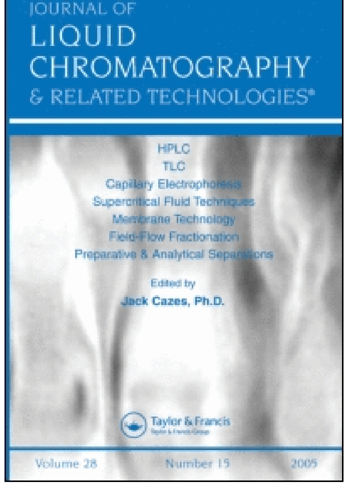

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JOURNAL OF LIQUID CHROMATOGRAPHY, 14(10), 1957-1966 (1991)

SEPARATION OF THE α-TOCOPHEROL MODEL COMPOUND 2,2,5,7,8-PENTAMETHYL-6-CHROMANOL FROM ITS OXIDATION PRODUCTS BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

A rapid method is described for the separation of the α-tocopherol model compound, 2,2,5,7,8-pentamethyl-6-chromanol (6), from 9 of its oxidation products in a single 35 minute run. Separated derivatives of 6, in order of elution, included the 5-cholesteroxymethyl (**1**), spirotrimer (**2**), spirodimer (**3**), 5-formyl (**4**), 5-ethoxymethyl (**5**), dihydroxydimer (**7**), chroman dione (**8**), quinone (**9**) and pyrano xanthene (**10**). A normal phase system, using gradient elution is employed, the eluent being monitored at 290 nm. The minimum detection limit for compounds **1-8** was 0.1 μg per injection and for compounds **9** and **10** it was 0.3 μg per injection.

INTRODUCTION

Of all the naturally occurring forms of vitamin E, α-tocopherol possesses the greatest biological activity (1). The major biological role of α-tocopherol is thought to

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be as a cellular antioxidant protecting membrane lipids from oxidation (2-4). Thus there is considerable interest in the oxidation chemistry of α-tocopherol (5-9,18,19). A great deal of this chemistry has been studied *in vitro* using the α-tocopherol model compound 2,2,5,7,8-pentamethyl-6-chromanol (**6**) (5-7,10,11) due to the crystalline nature of many of its oxidation products (as opposed to the oily products of α-tocopherol), as well as their simplified spectral properties. The absence of the phytyl side-chain in **6** has negligible effect on its *in vitro* antioxidative properties (12).

Many of the oxidation products of α-tocopherol and of **6** have been reported simultaneously (5-7). Quantitative analysis of these products can give valuable insight into the possible mode of action and metabolic fate of α-tocopherol *in vivo.* As yet however, while an abundance of chromatographic methods exist in the literature for the separation and quantitation of the naturally occurring tocopherols as found in foods, feeds and biological tissues (for extensive reviews, see 13, 14), there are very few methods describing the separation of α-tocopherol from its oxidation products. Most of these methods separate only a limited number of oxidation products (9,15,16). The most useful of those available is the HPLC method of Ha and Csallany (17) which separates α-tocopherol from 5 of its oxidation products. However, as the system is isocratic, the total analysis time is longer than that required by the present method. While those authors report the elution of α-tocopheryl quinone in 52 minutes, its analog, **9**, elutes in 25 minutes in our system. Further, they do not report the elution of compounds more polar than α-tocopheryl quinone. To date, there are few HPLC methods in the literature for the separation of **6** from a range of its oxidation products (18-20). Our studies have shown that oxidation of **6** gives a large number of products of which 8 occur frequently and do not appear to have been separated by HPLC. The aim of the present work was to devise an HPLC system which would separate these and other similar compounds.

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MATERIALS AND METHODS

Reagents and Solvents

Compound 6 and its oxidation products were prepared to satisfactory purity according to literature methods (Table 1). HPLC grade n-hexane, reagent grade chloroform and ethyl acetate were obtained from Ajax Chemicals (Sydney, Australia). Chloroform and ethyl acetate were doubly distilled before use.

Instrumentation and Method

Solvents [A n-hexane/chloroform (9:1 v/v); B n-hexane/chloroform/ethyl acetate (4:1:5 v/v/v)] were filtered (0.45 μm, Millipore) and degassed before use.

HPLC employed Waters Associates 501 (solvent A) and 6000A (solvent B) pumps, U6K injector, with 2 ml loop, a 660 solvent programer, an ETP Kortec K 95 variable wavelength detector (set at 290 nm for these experiments), a National Pen Recorder VP-6513, an SIC Chromatocorder 12 integrator and separations were performed by linear program (from 7 to 65 % solvent B in 0-40 min) on a LiChrosorb Si 60, 10 μm column (300 x 4.6 mm; packed in the School of Chemistry, University of New South Wales) at a flow rate of 1 mL/min.

Preparation of Standard Solutions

The ten standards were divided into three groups; (i) 5-cholesteroxymethyl-2,2,7,8-tetramethyl-6-chromanol (**1**). Compound **1** was kept separate from other

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TABLE 1

Oxidation Products of 2,2,5,7,8-Pentamethyl-6-chromanol (6) Separated by HPLC.

Systematic name	Number	Structure	Ref.	Abs. Max. (nm)
5-cholesteroxymethyl-2,2,7,8-tetramethyl-6-chromanol	1	HO, O, Chol	11	303, 298
spirotrimer of 6	2		21	292
spirodimer of 6	3		21	337, 299
5-formyl-2,2,7,8-tetramethyl-6-chromanol	4	HO, O, H	5	386,288,282,238
5-ethoxymethyl-2,2,7,8-tetramethyl-6-chromanol	5	HO, O	5	303, 298
2,2,5,7,8-pentamethyl-6-chromanol	6	HO	22	296
1,2-bis (2,2,7,8-tetramethyl-6-chromanol-5)-ethane	7	HO, OH	7	298, 293
2,2,7,8-tetramethylchroman-5,6-dione	8	O	23	438, 270
2-(3-methyl-3-hydroxybutyl)-3,5,6-trimethyl-1,4-benzoquinone	9	O, OH	23	268, 258
2,3-dihydro-3,3,5,6,9,10,11a(R)-heptamethyl-7a(S)-(3-hydroxy-3-methylbutyl)-1H-pyrano[2,3-a] xanthene-8-(7aH), 11(11aH)-dione	10	O, OH	6	297, 290, 250

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compounds as it was not always used as a standard. (ii) Chromanoid: spirotrimer of **6** (**2**), spirodimer of **6** (**3**), 5-formyl-2,2,7,8-tetramethyl-6-chromanol (**4**), 5-ethoxymethyl-2,2,7,8-tetramethyl-6-chromanol (**5**), **6** and 1,2-bis-(2,2,7,8-tetra-methyl-6-chromanol-5-)ethane (**7**) and (iii) quinonoid: 2,2,7,8-tetramethylchroman-5,6-dione (**8**), 2-(3-methyl-3-hydroxybutyl)-3,5,6-trimethyl-1,4-benzoquinone (**9**) and 2,3-dihydro-3,3,5,6,9,10,11a(R)- heptamethyl-7a(S)-(3-hydroxy-3-methylbutyl)-1H-pyrano[2,3-a] xanthene-8-(7aH),11(11aH)-dione (**10**). Chromanoid and quinonoid compounds were kept separate from each other to avoid any possibility of redox reactions occurring between them. All solutions were made up with solvent A and working concentrations were between 50 and 65 μg/mL.

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RESULTS AND DISCUSSION

Separation of **6** from 9 of its oxidation products is shown in Figure 1.

Compounds **2** and **3** predominate in oxidations which take place in non-polar solvents unless there is a reactive nucleophile such as an alcohol present in which case compounds such as **1** and **5** predominate. Compound **9** is always present to some degree but increases as the water concentration of the reaction increases. Compounds **4**, **7**, **8** and **10** would be regarded as secondary products.

The first five compounds elute within 6.5 minutes, thus the initial conditions had to be chosen carefully to prevent co-elution, but to keep the analysis time to a minimum. Minor impurities present in the standard solutions can be seen as 3 small peaks eluting just prior to **1**, **2** and after **8**.

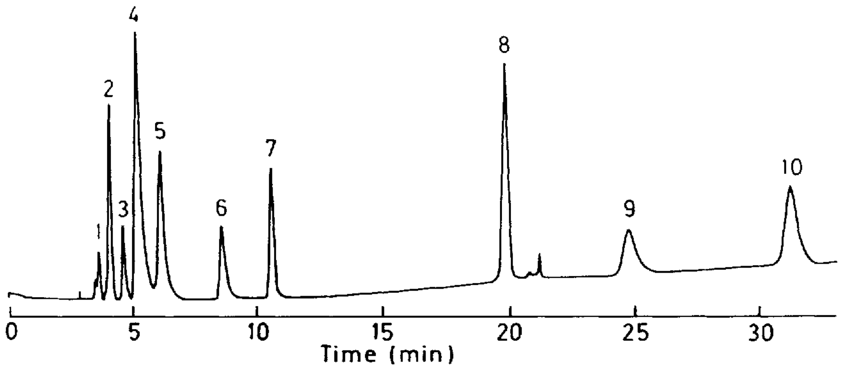

FIGURE 1.

HPLC separation of 2,2,5,7,8-pentamethyl-6-chromanol (6) from 9 of its oxidation products (as shown in Table 1). Gradient: Linear, 7-65%B over 0-40 min. Column: 300 x 4.6 mm (i.d.), LiChrosorb Si-60, 10 µm. Detection at 290 nm, range 0.32 AUFS. Solvent A: n-hexane/$CHCl_3$ (9:1); Solvent B: n-hexane/$CHCl_3$/ethyl acetate (4:1:5). Standard concentrations: approx. 30µg/ml.

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An unusual result was that 5-hydroxymethyl-2,2,7,8-tetramethyl-6-chromanol, one of the intermediates in the oxidation of **6**, was very difficult to detect by HPLC and the peaks were not reproducible. As it is very acid labile, it must be concluded that the silica column is sufficiently acidic to decompose it.

Choice of the detector wavelength was made after comparing the UV absorbing properties of **6** and its 9 oxidation products (Table 1). While the chromanoid compounds had maximum absorbance at approximately 290 nm, those containing the quinonoid chromophore had maximum absorbance (with a much greater extinction co-efficient) at approximately 270 or 250 nm.

After testing a range of detector wavelengths between 260 and 290 nm, the best single wavelength giving a good response for both chromanoid and quinonoid compounds was found to be 290 nm. The use of a higher wavelength also minimized the baseline drift caused by impurities in the ethyl acetate. At this wavelength, the minimum detection limits were approximately 0.1 μg per injection, except for **9** and **10** which were slightly higher at 0.3 μg due to their broader peak profile. While Ha and Csallany report a lower detection limit (17), the present method is convenient in that it uses a single detector wavelength throughout the analysis. In applications where greater sensitivity is required, it should be possible to use fluorescence detection, which, in addition to being more selective, has been reported (24) as 10 times more sensitive than UV absorbance in the analysis of tocopherols. However, detection of **9** and **10** would still require UV absorbance, although by combination with fluorescence detection, the UV wavelength could be specifically set to maximize the **8** and **9** response (270 nm), considerably improving their minimum detection limits.

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While the response factor linearity has not been measured over a wide range, all ten compounds gave a linear response over the range 0.3 - 1.3 μg per injection.

Earlier experiments in this laboratory (unpublished data) showed the same order of elution for the α-tocopherol analogs of **2**, **3**, **4**, **5**, **6**, **9** and **10** using isocratic elution with n-hexane/ethyl acetate (9:1 v/v). While lack of material has prevented those compounds from being run in this system, it may be possible to extend this method to the separation of α-tocopherol and its oxidation products.

In conclusion, this HPLC technique enables the rapid determination of **6** and its oxidation products in under 35 minutes. We have applied this method to study the rate of oxidation of **6** as a function of time, and these results will be published shortly.

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REFERENCES

1. Chow, C.K., Vitamin E in blood, Wld. Rev. Nutr. Diet., 45, 133, 1985.

2. Tappel, A.L., Vitamin E as the biological lipid antioxidant, Vitam. & Horm., 20, 493, 1962.

3. McCay, P.B., Fong, K.L., Lai, E. and King, M.M., Possible role of vitamin E as a free radical scavenger and singlet oxygen quencher in biological systems which initiate radical-mediated reactions, In "Tocopherols, Oxygen and Biomembranes", de Duve, C. and Hayaishi, O. (Eds), North-Holland and Biomedical press, Amsterdam, 1978, p. 41.

4. Urano, S. and Matsuo, M., Membrane stabilization of vitamin E, In "Clinical and Nutritional Aspects of Vitamin E", Hayaishi, O. and Mino, M. (Eds), Elsevier Science, B.V., 1987, p. 281.

5. Sumarno, M., Atkinson, E., Suarna, C., Saunders, J.K., Cole, E.R. and Southwell-Keely, P.T., Solvent influence on model oxidations of α-tocopherol, Biochim. et Biophys. Acta, 920, 247, 1987.

6. Suarna, C., Craig, D.C., Cross, K.J. and Southwell-Keely, P.T., Oxidations of vitamin E (α-tocopherol) and its model compound 2,2,5,7,8-pentamethyl-6-hydroxychroman, J. Org. Chem., 53, 1281, 1988.

7. Skinner, W.A. and Alaupovic, P., Oxidation products of vitamin E and its model compound 6-hydroxy-2,2,5,7,8-pentamethylchroman. V. Studies of the products of alkaline ferricyanide oxidation, J. Org. Chem., 28, 2854, 1963.

8. Ishikawa, Y. and Yuki, E., Reaction products from various tocopherols with trimethylamine oxide and their antioxidative activities, Agr. Biol. Chem., 39, 851, 1975.

9. Liebler, D.C., Baker, P.F. and Kaysen, K.L., Oxidations of vitamin E: evidence for competing oxidation and peroxyl radical trapping reactions of the tocopheroxyl radical, J. Am. Chem. Soc., 112, 6995, 1990.

10. Fujimaki, M., Kanamaru, K., Kurata, T. and Igarashi, O., Studies on the oxidation mechanism of vitamin E. Part 1. The oxidation of 2,2,5,7,8-pentamethyl-6-hydroxychroman, Agr. Biol. Chem., 34, 1781, 1970.

11. Suarna, C. and Southwell-Keely, P.T., Effects of alcohols on the oxidation of the vitamin E model compound 2,2,5,7,8-pentamethyl-6-chromanol, Lipids, 24, 56, 1989.

12. Nilsson, J.L.G., Studies of tocopherols and related chromanols, Acta Pharm. Suec., 6, 1, 1969.

13. Nelis, H.J., De Bevere, V. and De Leenheer, A.P., Vitamin E: tocopherols and tocotrienols, In "Chromatographic Science ", De Leenheer, A.P., Lambert, W.A. and De Ruyter, M.G.M., (Eds), Vol. 30, Marcel Dekker, N.Y., 1985, p. 129.

14. Ball, G.F.M., Fat soluble vitamin assays in food analysis: a comprehensive review, Elsevier Applied Science, London, 1988, p. 117

15. Yamauchi, R., Kato, K. and Ueno, Y., Formation of trimers of α-tocopherol and its model compound 2,2,5,7,8-pentamethyl-6-chromanol in autoxidising methyl linoleate, Lipids, 23, 779, 1988.

16. Kaiser, S., Di Mascio, P., Murphy, M.E. and Sies, H., Physical and chemical scavenging of singlet molecular oxygen by tocopherols, Arch. Biochem. Biophys., 277, 101, 1990.

17. Ha, Y.L. and Csallany, A.S., Separation of α-tocopherol and its oxidation products by high performance liquid chromatography, Lipids, 23, 359, 1988.

18. Matsuo, M., Matsomoto, S., Iitaka, Y. and Niki, E., Radical scavenging reaction of vitamin E and its model compound, 2,2,5,7,8-pentamethylchroman-6-ol in a tert-butylperoxyl radical generating system, J. Am. Chem. Soc. 111, 7179, 1989.

19. Matsuo, M., Matsumoto, S. and Iitaka, Y., Oxygenations of vitamin E (α-tocopherol) and its model compound, 2,2,5,7,8-pentamethylchroman-6-ol, in the presence of superoxide radical solubilized in aprotic solvents: Unique epoxidations and recyclizations. J. Org. Chem. 52, 3514 1987.

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20. Ozawa, T. and Hanaki, A., Free radicals of tocopherol model compound 6-hydroxy-2,2,5,7,8-pentamethylchroman which are produced from the reaction with superoxide ion O_2^-: Studies by HPLC. Biochem. Biophys. Res. Comm. 129, 461 1985.

21. Schudel, P., Mayer, H., Metzger, L., Ruegg, R. and Isler, O., Über die chemie des vitamin E. Die struktur des kalium ferricyanid oxydations produktes von α-tokopherol, Helv. Chim. Acta, 46, 636, 1963.

22. Smith, L.I., Ungnade, H.E., Hoehn, H. and Wawzonek, S., The chemistry of vitamin E. VI. The addition of dienes to phenols and hydroquinones, J. Org. Chem., 4, 311, 1939.

23. John, W., Dietzel, E. and Emte, W., Über einige oxydationsprodukte der tokopherole und analoger einfacher modellkörper. 6. Mitteilung über antisterilitätsfaktoren (vitamin E), Z. Physiol. Chem., 257, 173, 1939.

24. Thompson, J.N. and Hatina, G., Determination of tocopherols and tocotrienols in foods and tissues by high performance liquid chromatography, J. Liq. Chromat., 2, 327, 1979.

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