

7c and 7d) but are consistent with the line XX' moving closer to the line RS. In particular, over and above general solvent broadening, bands 1, 2 and 3 become slightly narrower: this is consistent with the observations on the unlabeled complex **1** shown in Figure 2.

### Conclusions

As far as we are aware, this is the first example of a compound that illustrates clear IR coalescence which can be predicted on the basis of a very simple model. The complicated  $\nu(\text{CO})$  spectrum of 42%  $^{13}\text{C}$ -enriched  $\text{Fe}(\text{CO})_3(\eta^4\text{-norbornadiene})$  in LKr/Xe at low temperatures can be accurately fitted with the four parameters considered in the energy-factored CO force field appropriate for the  $\text{M}(\text{CO})_3$  group under  $C_s$  symmetry. Warming the molecule to high temperature in scXe produces dramatic spectral changes including band broadening and coalescence. The appearance of the high-temperature "rapid exchange" spectrum can be predicted using the two parameters appropriate for the  $\text{M}(\text{CO})_3$  group under (pseudo)- $C_{3v}$  symmetry, which are obtained by statistically averaging the above four parameters.

We are aware that this is not the complete story, but only the first step toward the final goal of extracting kinetic data from variable-temperature IR spectra, which would then allow the evaluation of activation parameters for the exchange process. On the other hand, it would be very appealing to establish dynamic IR spectroscopy as a tool for gaining insight into very fast processes, associated with extremely low barriers, far below the 5–6-kcal mol<sup>-1</sup> accessible by dynamic NMR spectroscopy. But, on the other hand, such a low barrier makes the potential wells very flat, and in consequence of this there can be some contribution to the spectra from molecules in higher levels within the potential wells and above the exchange barrier. Details of these considerations and calculations will be published elsewhere.

### Experimental Section

$\text{Fe}(\text{CO})_3(\eta^4\text{-norbornadiene})$  (**1**) and  $\text{Fe}(\text{CO})_3(\eta^4\text{-butadiene})$  (**2**) were synthesized according to established procedures.<sup>21</sup> Isotopically labeled carbon monoxide (99%  $^{13}\text{C}$ , <1%  $^{18}\text{O}$ ; CLM-1845, Cambridge Isotope Laboratories, Woburn/MA, USA) was purchased from Promochem (Wesel/Germany). The  $^{13}\text{C}$  enrichment of **1** was performed by irradiation of 0.2 g of the complex in *n*-pentane (50 mL) under  $^{13}\text{C}$  atmosphere (70 mL, ca. 1.2 bars) until the high-frequency band of the starting material was reduced in intensity by a factor of ca. 5. After evaporation under vacuum, the residual solution (ca. 3 mL) was cooled to dry ice temperature, whereupon yellow crystals precipitated and were separated from the supernatant solution and dried under vacuum. Mass spectral analysis showed the following composition of the  $\text{Fe}(\text{CO})_3(\eta^4\text{-norbornadiene})$  sample: 19% ( $n = 0$ ), 43% ( $n = 1$ ), 31% ( $n = 2$ ), and 7% ( $n = 3$ ). Krypton and xenon (BOC Research Grade) were used without further purification. The cells for IR spectroscopy in liquid noble gas solvents at low temperature and in supercritical fluids have been described elsewhere.<sup>9,10</sup> Spectra in these solvents were run on either Nicolet 7199 (with MX-3600 data station) or Nicolet 730 FTIR instruments. The spectra in hydrocarbon solvents were run on a Perkin-Elmer 1760 FTIR instrument (with PE 7500 data station) using a low-temperature cell (1-mm pathlength,  $\text{CaF}_2$  windows, cooled with nitrogen gas), which will be described elsewhere.<sup>22</sup>

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(21) For a review see: King, R. B. In *The Organic Chemistry of Iron*; Koerner von Gustorf, E. A., Grevels, F.-W., Fischler, I., Eds.; Academic Press: New York, 1987, Vol. 1, p 525.

(22) Klotzbücher, W. E. Manuscript in preparation.

## Dye-Sensitized Photooxidation of Vitamin E Revisited. New 7-Oxaspiro[4.5]dec-1-ene-3,6-dione Products by Oxygenation and Ring Contraction of $\alpha$ -Tocopherol

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**Abstract:** The dye-sensitized photooxidation of  $\alpha$ -tocopherol, the major component of the biological antioxidant vitamin E, was reinvestigated under biomimetic conditions. When methylene blue was used as the sensitizer and ethanol-phosphate buffer (pH 6.8; 7:3, v/v) as the solvent, the reaction led to the formation, besides  $\alpha$ -tocopherol quinone (**3**) and its epoxide (**4**), of three hitherto unknown photooxygenation products accounting overall for about 15% of the consumed substrate. These were identified as two diastereoisomers corresponding to the gross structure of 1,2,8-trimethyl-4-methylene-8-phytyl-7-oxaspiro[4.5]dec-1-ene-3,6-dione (**8**) and one diastereomer of the hydrated derivative 4-hydroxy-1,2,4,8-tetramethyl-8-phytyl-7-oxaspiro[4.5]dec-1-ene-3,6-dione (**9**). Formation of the 7-oxaspiro[4.5]dec-1-ene-3,6-dione system was envisaged as resulting from a ring-contracting rearrangement of an epoxyquinone hemiketal intermediate (**10**) or a related species arising from singlet oxygenation of  $\alpha$ -tocopherol.

### Introduction

Vitamin E, which occurs mainly in the form of  $\alpha$ -tocopherol, occupies a central position in the defense armamentarium of living cells against lipid peroxidation and membrane damage accompanying oxidative stress conditions.<sup>1,2</sup> The biological activity of  $\alpha$ -tocopherol stems primarily from the ability to act as an efficient

chain-breaking antioxidant, trapping reactive peroxy radicals and competing with oxidizable unsaturated lipids during aerial oxidation processes.<sup>3,4</sup>

There is, in addition, more than suggestive evidence that  $\alpha$ -tocopherol exerts a fundamental protective function against those

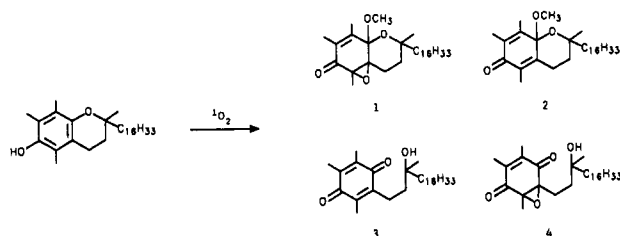
(1) Machlin, L. J. *Vitamin E: A Comprehensive Treatise*; Marcel Dekker: New York, 1980.

(2) Green, J.; McHale, D. In *Biochemistry of Quinones*; Morton, R. A., Ed.; Academic Press: London, 1965; pp 261–285.

(3) Burton, G. W.; Ingold, K. U. *Acc. Chem. Res.* **1986**, *19*, 194–201 and references cited therein.

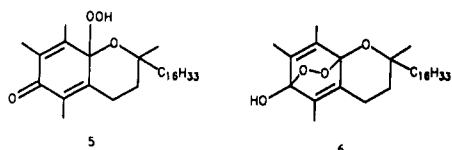
(4) Kagan, V. E.; Bakalova, R. A.; Zhelev, Zh. Zh.; Rangelova, D. S.; Serbinova, E. A.; Tyurin, V. A.; Denisova, N. K.; Packer, L. *Arch. Biochem. Biophys.* **1990**, *280*, 147–152.

Scheme I

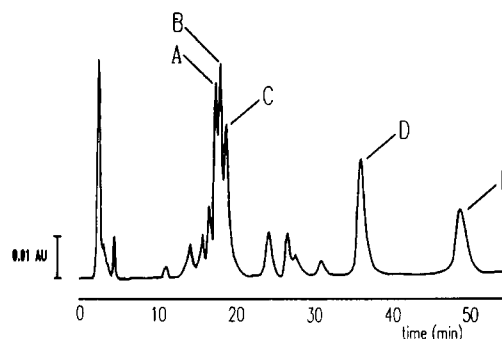


specific cell-damaging processes which are under the influence of light and sensitizing pigments and are mediated by singlet oxygen,<sup>3-7</sup>  $^1\Delta_g$ . These latter processes have been increasingly implicated to account for the apparent relationship between chronic solar exposure and the onset of pathological conditions, especially skin cancer.<sup>8-10</sup> As a consequence, considerable efforts have been devoted over the past 20 years to elucidate the mechanism of interaction of  $\alpha$ -tocopherol with singlet oxygen. From all these studies, it is now clear that  $\alpha$ -tocopherol is a highly efficient scavenger of singlet oxygen, both by physical quenching and by chemical reaction. The balance of these processes depends on the properties of an initially formed exciplex,<sup>11</sup> to which three options are offered: reversion to reactants, collapse to ground states via intersystem crossing, and evolution to products. As a rule, physical quenching predominates, but chemical oxidation is by no means a negligible process, at least in polar solvents. The nature of the products of the singlet oxygen oxidation of  $\alpha$ -tocopherol was first investigated by Grams et al.<sup>12,13</sup> in a study of the dye-sensitized photooxidation reaction in methanol. These researchers reported the formation of 4a,5-epoxy-8a-methoxy- $\alpha$ -tocopherone (1) and 8a-methoxy- $\alpha$ -tocopherone (2) as the major reaction products, along with lower amounts of  $\alpha$ -tocopherolquinone (3) and its 2,3-epoxide (4) (Scheme I). Interestingly, the relative proportions of these compounds were found to be quite different when singlet oxygen was generated chemically by hypochlorite oxidation of hydrogen peroxide.<sup>14</sup> Under these conditions, 3 and 4 were prevailing over 1 and 2.

Subsequent work by Foote and his associates<sup>15</sup> provided evidence that the dye-sensitized reaction leads to the formation in the early stages of the modestly stable hydroperoxydienone 5, which on standing, is smoothly converted to 3 and 4. This finding was taken as evidence against the intermediacy of endoperoxide adducts of the type 6, originally invoked by Grams et al. to account for the formation of products 1-4.



Recently, as a part of a research program on the photochemistry of psoralens and related skin photosensitizing furocoumarins,<sup>16</sup>



**Figure 1.** HPLC elution profile of the main products formed by dye-sensitized photooxidation of  $\alpha$ -tocopherol. Annotations identify compounds 3, 4, 8, and 9 as specified in the text.

we found that these compounds were able to sensitize  $\alpha$ -tocopherol photooxidation in ethanol-phosphate buffer with pyrex-filtered UV light.<sup>17</sup> Analysis of the reaction mixture provided evidence for a complex pattern of oxidation products of  $\alpha$ -tocopherol, some of which exhibited chromatographic and spectral properties that, to the best of our knowledge, did not match those of any of the products so far reported in the literature. Interestingly enough, the same products were obtained by conventional dye-sensitized photooxidation with methylene blue in ethanol-phosphate buffer. This finding prompted us to reexamine in detail the nature of the products formed by dye-sensitized photooxidation of  $\alpha$ -tocopherol. Careful chromatographic fractionation of the mixture led to the isolation, besides 3 and 4, of three products, which were shown to contain a hitherto unknown 7-oxaspiro[4.5]dec-1-ene-3,6-dione structure arising by oxidative ring contraction of the chroman system of  $\alpha$ -tocopherol.

## Results and Discussion

$\alpha$ -Tocopherol was photooxidized in oxygen-saturated ethanol-phosphate buffer at pH 6.8 (7:3, v/v) in an ice-water bath with methylene blue as sensitizer. Figure 1 shows a reverse-phase HPLC profile of the reaction mixture after 3 h of irradiation. A complex pattern of photooxidation products was formed, comprising, besides Grams' compounds 3 and 4, corresponding to the peaks designated E and D, a series of products with shorter retention times, three of which, corresponding to peaks A, B, and C, were predominant and better resolved. These latter could be obtained as colorless oils, homogeneous to HPLC and TLC, after careful fractionation of the reaction mixture by combined flash<sup>18</sup> and preparative thin-layer chromatography on silica.

The most abundant products, corresponding to peaks eluted after 18.6 (B) and 19.4 (C), displayed nearly identical UV spectra, with a maximum at 238 nm. FT-IR spectra showed two carbonyl stretching bands at 1715 and 1708  $\text{cm}^{-1}$  (B) and 1720 and 1706  $\text{cm}^{-1}$  (C).

The structural analogy of these compounds was also apparent from NMR analysis. The proton spectra exhibited, besides complex overlapping signals in the region between  $\delta$  0.8 and 2.0, due to the aliphatic  $\text{C}_{16}\text{H}_{33}$  chain, two 1 H singlets at  $\delta$  5.46 and 6.07 for both compounds, and three methyl resonances at  $\delta$  1.50, 1.84, and 2.06 (B) and  $\delta$  1.48, 1.83, and 2.07 (C). When compared to the  $^1\text{H}$  NMR features of tocopherol, these data indicate that one of the four methyl groups originally present had been replaced in the product by an exocyclic methylene group. Scrutiny of the broad-band decoupled  $^{13}\text{C}$  NMR spectra, aided by DEPT multiplicity discrimination,<sup>19</sup> suggested the existence in both B and C of a conjugated carbonyl group ( $\delta$  193.9), an ester (or lactone) carbonyl ( $\delta$  170.5 for B and 170.4 for C), a fully substituted double bond adjacent to the carbonyl ( $\delta$  138.9 and 166.5 for B, 138.8 and 166.7 for C), an unsubstituted exocyclic double bond ( $\delta$  114.2

(5) Foote, C. S.; Ching, T.-Y.; Geller, G. G. *Photochem. Photobiol.* **1974**, *20*, 511-513.

(6) Stevens, B.; Small, R. D.; Perez, S. R. *Photochem. Photobiol.* **1974**, *20*, 515-517.

(7) Fahrenholz, S. R.; Doleiden, F. H.; Trozzolo, A. M.; Lamola, A. A. *Photochem. Photobiol.* **1974**, *20*, 505-509.

(8) Foote, C. S. In *Free Radicals in Biology*; Pryor, W. A., Ed.; Academic Press: New York, 1976; Vol. II, pp 85-133.

(9) Kochevar, I. E.; Gange, R. W. *Photochem. Photobiol.* **1983**, *37*, 695-700.

(10) Kantor, G. J. *Photochem. Photobiol.* **1985**, *41*, 741-746.

(11) Gorman, A. A.; Gould, I. R.; Hamblett, I.; Standen, M. C. *J. Am. Chem. Soc.* **1984**, *106*, 6956-6959.

(12) Grams, G. W.; Eskins, K.; Inglett, G. E. *J. Am. Chem. Soc.* **1972**, *94*, 866-868.

(13) Grams, G. W.; Eskins, K. *Biochemistry* **1972**, *11*, 606-608.

(14) Grams, G. W. *Tetrahedron Lett.* **1971**, 4823-4825.

(15) Clough, R. L.; Yee, B. G.; Foote, C. S. *J. Am. Chem. Soc.* **1979**, *101*, 683-686.

(16) d'Ischia, M.; Napolitano, A.; Prota, G. *Biochim. Biophys. Acta* **1989**, *993*, 143-147.

(17) Costantini, C.; d'Ischia, M.; Misuraca, G.; Prota, G. Manuscript in preparation.

(18) Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923-2925.

(19) Doddrell, D. M.; Pegg, D. T.; Bendall, M. R. *J. Magn. Reson.* **1982**, *48*, 323-327.

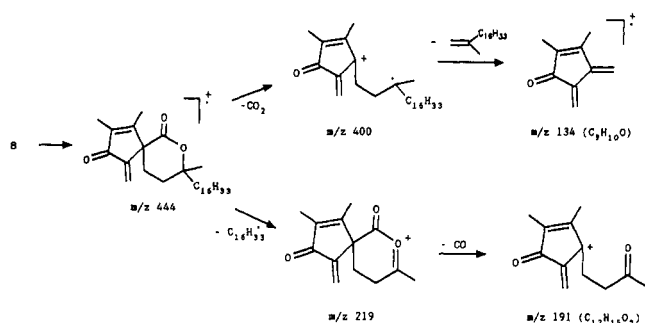
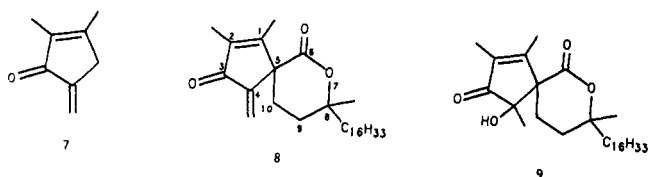


Figure 2. Proposed mechanism for the mass spectral fragmentation of 8.

and 149.5 for B, 114.0 and 149.4 for C), and two deshielded quaternary aliphatic carbons ( $\delta$  54.5 and 86.2 for B, 54.7 and 86.1 for C). These data, coupled with the striking similarity of certain spectral features of B and C (UV, IR, portions of the  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra) to those of the antibiotic methylenomycin B (7),<sup>20</sup> allowed formulation of compounds B and C as 1,2,8-trimethyl-4-methylene-8-phytyl-7-oxaspiro[4.5]dec-1-ene-3,6-dione (8).



Most of the carbon-carbon connections in 8 were confirmed by long-range 2D carbon-proton shift correlation experiments,<sup>21</sup> using delay  $D_3 = 71$  ms (corresponding to  $J_{\text{C,H}} = 7$  Hz). In particular, the following C-H correlations were clearly detected for compound C:  $^2J$  coupling between the C1-CH<sub>3</sub> protons ( $\delta$  2.07) and the C1 carbon ( $\delta$  166.7), the C2-CH<sub>3</sub> protons ( $\delta$  1.83) and the C2 carbon ( $\delta$  138.8), and the C8-CH<sub>3</sub> protons ( $\delta$  1.48) and the C8 carbon ( $\delta$  86.1);  $^3J$  coupling between the C4-CH<sub>2</sub> protons ( $\delta$  5.46, 6.07) and the C3 carbonyl carbon ( $\delta$  193.9), the C1-CH<sub>3</sub> protons ( $\delta$  2.07) and the C2 ( $\delta$  138.8) and C5 ( $\delta$  54.7) carbons; the C2-CH<sub>3</sub> protons ( $\delta$  1.83) and the C1 carbon ( $\delta$  166.7), and the C4-CH<sub>2</sub> proton ( $\delta$  6.07) and the C5 carbon ( $\delta$  54.7).

Further evidence supporting structure 8 came from analysis of the EI mass spectra of B and C, which were virtually identical and were consistent with a molecular formula of  $\text{C}_{29}\text{H}_{48}\text{O}_3$ . A rationale for the fragmentation pattern of 8 is shown in Figure 2. Generation of a base peak at  $m/z$  134 is envisaged as due to sequential loss of carbon dioxide and a neutral fragment containing the phytol side chain, whereas formation of a weak peak at  $m/z$  191 is taken as evidence for a minor fragmentation route involving losses of the phytol side chain and carbon monoxide. High-resolution measurement of ions at  $m/z$  134 (found 134.0728, exact mass calcd for  $\text{C}_9\text{H}_{10}\text{O}$  134.0731) and 191 (found 191.1072, exact mass calcd for  $\text{C}_{12}\text{H}_{15}\text{O}_2$  191.1078) confirmed the elemental composition of the relevant fragments proposed in Figure 2.

Compounds B and C represent two diastereomeric pairs of enantiomers<sup>22</sup> differing in the relative configurations at C5 and C8. Attempts to assign the correct stereochemical relationships by analysis of the  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra were unsuccessful. NOE difference experiments proved likewise inconclusive.

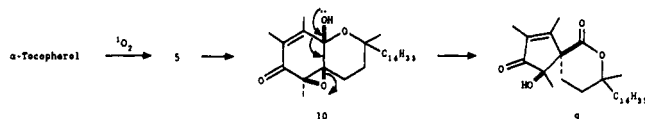


Figure 3. Suggested mechanism for the formation of 9 by singlet oxygenation of  $\alpha$ -tocopherol. Plausible stereochemical relationships between the newly developed asymmetric centers are highlighted.

The product eluted after 18.0 min (A) gave a parent ion in the EIHRMS at  $m/z$  462.3680, consistent with a molecular formula of  $\text{C}_{29}\text{H}_{50}\text{O}_4$ , differing from the molecular formula of B and C simply by addition of  $\text{H}_2\text{O}$ . The UV spectra showed a maximum at 232 nm. Main features of the FT-IR spectrum were broad OH stretching bands at 3555 and 3418  $\text{cm}^{-1}$  and a broad carbonyl band centered at 1718  $\text{cm}^{-1}$ . Analysis of the  $^1\text{H}$  NMR spectrum showed no signal in the  $\text{sp}^2$  region, and four deshielded methyl groups, resonating at  $\delta$  1.31, 1.43, 1.77, and 2.03, besides the usual signals of the phytol chain. These data were suggestive of a structural relationship between A and the B-C pair, whereby the exocyclic double bond in the latter was suppressed by addition of the elements of water, restoring the set of four methyl groups pertaining to the original chroman nucleus. Evidence supporting this view came from the  $^{13}\text{C}$  NMR spectrum, in which the carbonyl resonance was shifted downfield to  $\delta$  206.9, as a result of loss of the conjugated exocyclic double bond, and a new signal at  $\delta$  78.3, ascribable to an oxygen-bearing aliphatic quaternary carbon, was apparent. Other salient features of the spectrum included the resonances ( $\delta$  134.1 and 168.8) of the carbonyl-connected, fully substituted double bond and the aliphatic signals at  $\delta$  60.3 and 85.7, due to two quaternary carbons. Accordingly, A was assigned structure 9.

Structure 9 is marked by the presence in the 7-oxaspiro-[4.5]dec-6-ene ring system of the three chiral centers, implying that four diastereomeric pairs of enantiomers can in principle be formed (neglecting the phytol chain).<sup>22</sup> Yet, no evidence was apparently obtained for such a mixture of isomers. Though it may not be ruled out, considering the complexity of the oxidation mixture, that some diastereoisomers have escaped our analysis, there are grounds to believe that one diastereoisomer (actually one pair of enantiomers) is prevailing. Any explanation of this intriguing finding would be speculative, inasmuch as all attempts to investigate the relative stereochemistry of the chiral centers by spectral techniques, including NOE difference experiments, did not provide conclusive information.

Products 8 and 9 apparently escaped the attention of previous workers on the dye-sensitized photooxidation of  $\alpha$ -tocopherol. The reason lies probably in the solvents used by these researchers, i.e., methanol and chloroform, which contained negligible amounts of water. Indeed, when the methylene blue sensitized photooxidation was carried out in dry methanol, no significant amount of products 8 and 9 could be detected when all the substrate was consumed. However, addition of 20% phosphate buffer restored the product pattern observed with ethanol-phosphate buffer. Apparently, the yields of 3 and 4 were not dependent on the presence of water.

The formation of products 8 and 9 by methylene blue sensitized photooxidation of  $\alpha$ -tocopherol is at first sight not straightforward. The dye, oxygen, and visible light were clearly required, suggesting the involvement of singlet oxygen as the actual oxidizing species. Moreover, tocopherol quinone (3) and the quinone epoxide 4 were apparently not involved in the pathway leading to 8 and 9, since when photooxidized under the experimental conditions used for  $\alpha$ -tocopherol, the pure compounds, prepared by a chemical procedure, were not converted into 8 and/or 9. A possible mechanism accounting for the formation of 9 is schematically outlined in Figure 3. In this, the key event is a rearrangement suffered by an epoxy quinone hemiketal intermediate (10). The driving force of the ring contraction would be provided by the formation of the thermodynamically stable, spirofused cyclopentenone and  $\delta$ -lactone rings. Formation of the intermediate 10 is supposed to follow from Foote's hydroperoxydienone by a route that is competitive to ring opening, yielding tocopherolquinone 3. An interesting implication

(20) Tius, M. A.; Astrab, D. P.; Fauq, A. H.; Ousset, J. B.; Trehan, S. J. *Am. Chem. Soc.* **1986**, *108*, 3438-3442.

(21) Freeman, R.; Morris, G. A.; *J. Chem. Soc., Chem. Commun.* **1978**, 684-686.

(22) The use of *dl*- $\alpha$ -tocopherol, which is also reported in the papers by Grams<sup>12-14</sup> and Foote,<sup>15</sup> would imply in principle the formation of at least eight stereoisomers (four diastereoisomers) for each product, isomerism being determined by the three asymmetric carbons on the phytol chain<sup>23</sup> plus any chiral center within the chroman domain. Yet, in the present study, as well as in previous papers, only diastereoisomeric products that differ in the stereochemistry of the chroman-derived moiety could apparently be discriminated.

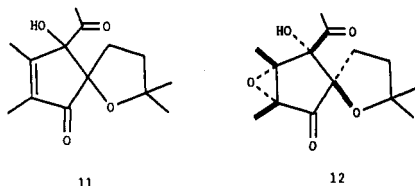
(23) Cohen, N.; Scott, C. G.; Neukom, C.; Lopresti, R. J.; Weber, G.; Saucy, G. *Helv. Chim. Acta* **1981**, *64*, 1158-1173.

of the proposed mechanism is that the relative configuration of the chiral centers at C4 and C5 in structure **9** would be imposed by the mode of attack of the migrating carbon-carbon bond in **10**. On the reasonable assumption that in **10** the hydroxyl and epoxy groups lie on the same side, this attack would displace the epoxide from the rear, causing the resulting hydroxyl at C4 and the lactone carbonyl to be spatially close.

Under the reaction conditions of  $\alpha$ -tocopherol photooxidation, **9** was not converted into **8** to any significant extent, suggesting that this latter structure does not arise from simple dehydration of **9**, but is formed through a different route.

We are unable, with the information now available, to propose explanations that account for the observed effect of water on the reaction course. The tentative mechanism outlined in Figure 3, while useful in rationalizing the origin of **9**, ignores a number of factors, including the role of water, that may be important in determining the nature of the products formed. Several other facets of the proposed mechanism also await verification, including the actual nature of the intermediates and the stereochemistry of the ring-contraction process.

Apart from the above mechanistic considerations, the formation of spiroactone products by dye-sensitized photooxidation of  $\alpha$ -tocopherol is of interest, as it sheds light on a previously unrecognized aspect of the reactivity of the 6-hydroxychroman system. Products having a spirofused bicyclic carbon skeleton are not unprecedented in  $\alpha$ -tocopherol chemistry. Oxidation of the model compound 2,2,5,7,8-pentamethylchroman-6-ol with superoxide ions in aprotic solvent has recently been reported<sup>24,25</sup> to give rise, *inter alia*, to the ring-contracted products **11** and **12**. Yet, no products of this kind have so far been isolated under photooxidative conditions.



Work is now in progress to assess the biological properties of the new products and their occurrence in natural sources.

## Experimental Section

**General Procedures.** *dl*- $\alpha$ -Tocopherol and methylene blue were purchased from Fluka. All other chemicals and solvents were of the highest quality available and were used without further purification.  $\alpha$ -Tocopherolquinone (**3**) and the epoxide **4** were prepared by a procedure similar to that reported by Grams,<sup>14</sup> involving oxidation of  $\alpha$ -tocopherol with chemically generated singlet oxygen.

UV spectra were determined with a Perkin-Elmer Lambda 7 spectrophotometer. FT-IR spectra were recorded on a Perkin-Elmer Model 1760-X spectrophotometer. EIMS and high-resolution mass spectra were determined with a Kratos MS 50 spectrometer. Samples were ionized with a 70-eV electron beam. Main fragmentation peaks are reported with their relative intensity (percent values are in brackets).

<sup>1</sup>H NMR (270 or 400 MHz) and <sup>13</sup>C NMR (67.9 or 100 MHz) spectra were carried out on a Bruker AC 270 or on a Bruker WM 400 spectrometer equipped with an Aspect 3000 computer. Spectra were recorded in acetone-*d*<sub>6</sub> with tetramethylsilane as reference standard. Polarization transfer (DEPT) experiments<sup>19</sup> were performed by using a polarization transfer pulse of 135° and a delay adjusted to an average C-H coupling of 125 Hz. Long-range 2D carbon-proton shift correlation experiments<sup>21</sup> were performed at 100 MHz. A Bruker XHCORR microprogram with delay *D*<sub>3</sub> = 71 ms was used. Experiments were recorded with 256 × 1024 data matrix sizes.

Analytical and preparative HPLC were performed by using a Gilson Model 302 pump, a Gilson 316 UV detector, and a 4 × 250 mm RP 18 Lichrochart cartridge (Merck) or a 10 × 250 mm RP 18 Lichrosorb column. Detection was carried out at  $\lambda$  254 nm.

Analytical and preparative TLC were carried out on precoated silica gel F-254 plates (0.25- and 0.50-mm layer thickness, Merck). Flash column chromatography utilized silica gel 60 (230–400-mesh ASTM, Merck). Proportions for mixed solvent are by volume.

**Dye-Sensitized Photooxidation of  $\alpha$ -Tocopherol.** An ice-water-cooled mixture of *dl*- $\alpha$ -tocopherol (860 mg,  $2.0 \times 10^{-3}$  mol) and methylene blue (4 mg) in 2.0 L of ethanol-phosphate buffer (0.02 M, pH 6.8; 7:3, v/v) was irradiated externally with a 500-W tungsten lamp, with oxygen bubbling through the solution continuously. The disappearance of  $\alpha$ -tocopherol was monitored by HPLC using methanol as the eluant, at a flow rate of 1.5 mL/min. The reaction mixture was analyzed by HPLC with methanol/water (9:1) as the eluant, at a flow rate of 1.5 mL/min. When most of the substrate had disappeared, HPLC analysis revealed the presence of a complex pattern of products, five of which, designated A–E, accounted for most of the photooxidized tocopherol. They eluted at 18.0, 18.6, 19.4, 35.8, and 48.8 min, in the order. After 3 h of irradiation, the reaction mixture was concentrated to about 300 mL in a rotary evaporator under reduced pressure and then extracted with ethyl acetate. The organic layer was dried over Na<sub>2</sub>SO<sub>4</sub> and evaporated to dryness to give about 800 mg of a brownish oil. This was dissolved in light petroleum and fractionated by flash chromatography<sup>18</sup> on a silica gel column (40 × 4 cm). After washing with 2 L of light petroleum-ethyl ether (95:5), to remove faster moving products (60 mg) and the unreacted  $\alpha$ -tocopherol (53 mg), a major fraction (581 mg) was eluted with 650 mL of light petroleum-ethyl ether (4:6). This fraction was chromatographed on preparative silica gel plates (ethyl acetate-cyclohexane (25:75)) to afford four main distinct UV-detectable bands at *R*<sub>f</sub> = 0.65, 0.54, 0.48, and 0.42. The first band (260 mg) proved to consist of two products coeluting with peaks E and D on HPLC. These were isolated by preparative HPLC (eluant, methanol/1 M sodium acetate (pH 4.25; 93:7, v/v), flow rate 6 mL/min) as colorless oils, and were identified as  $\alpha$ -tocopherolquinone (**3**; 67 mg) and its epoxide (**4**; 180 mg) by spectral analysis (NMR, EIMS, UV) and comparison of the chromatographic properties with those of authentic samples.

The bands at *R*<sub>f</sub> = 0.54 and 0.48, corresponding to peaks B and C on HPLC, afforded two diastereoisomers of 1,2,8-trimethyl-4-methylene-8-phytyl-7-oxaspiro[4.5]dec-1-ene-3,6-dione (**8**).

Data for B: colorless oil (41 mg); MS (EI) *m/z* 444 (16), 400 (41), 219 (10), 191 (19), 134 (100); exact mass calcd for C<sub>29</sub>H<sub>48</sub>O<sub>3</sub> 444.3603, found 444.3582; UV (cyclohexane)  $\lambda$  238 nm; FTIR (CCl<sub>4</sub>) 2955, 2930, 2870, 1715, 1708, 1630 cm<sup>-1</sup>; <sup>1</sup>H NMR (selected resonances)<sup>26</sup>  $\delta$  1.50 (s, 3 H, C8-CH<sub>3</sub>), 1.84 (s, 3 H, C2-CH<sub>3</sub>), 2.06 (s, 3 H, C1-CH<sub>3</sub>), 5.46 (s, 1 H, C4-CH<sub>2</sub>) 6.07 (s, 1 H, C4-CH<sub>2</sub>); <sup>13</sup>C NMR  $\delta$  8.1, 13.1, 19.7, 19.7, 19.8, 21.9, 22.6, 22.7, 24.9, 25.2, 27.2, 28.3, 29.6, 33.0, 33.1, 33.2, 37.7, 37.8, 39.8, 41.2, 41.3, 54.5, 86.2, 114.2, 138.9, 149.5, 166.5, 170.5, 193.9.

Data for C: colorless oil (56 mg); MS (EI) *m/z* 444 (2), 400 (18), 219 (3), 191 (8), 134 (100); exact mass calcd for C<sub>29</sub>H<sub>48</sub>O<sub>3</sub> 444.3603, found 444.3610; UV (cyclohexane)  $\lambda$  238 nm; FTIR (CCl<sub>4</sub>) 2950, 2930, 2870, 1720, 1706, 1630 cm<sup>-1</sup>; <sup>1</sup>H NMR (selected resonances)<sup>26</sup>  $\delta$  1.48 (s, 3 H, C8-CH<sub>3</sub>), 1.83 (s, 3 H, C2-CH<sub>3</sub>), 2.07 (s, 3 H, C1-CH<sub>3</sub>), 5.46 (s, 1 H, C4-CH<sub>2</sub>) 6.07 (s, 1 H, C4-CH<sub>2</sub>); <sup>13</sup>C NMR  $\delta$  8.1, 12.9, 19.7, 19.7, 19.8, 21.4, 22.6, 22.7, 24.9, 25.2, 25.7, 28.6, 29.8, 33.2, 33.2, 33.4, 37.7, 37.8, 37.8, 39.8, 43.8, 54.7, 86.1, 114.0, 138.8, 149.4, 166.7, 170.4, 193.9.

The band at *R*<sub>f</sub> = 0.42, corresponding to peak A on HPLC, afforded one diastereomer of 4-hydroxy-1,2,4,8-tetramethyl-8-phytyl-7-oxaspiro[4.5]dec-1-ene-3,6-dione (**9**): colorless oil (33 mg); MS (EI) *m/z* 462 (18), 418 (99), 400 (100), 237 (70); exact mass calcd. for C<sub>29</sub>H<sub>50</sub>O<sub>4</sub> 462.3709, found 462.3680; UV (cyclohexane)  $\lambda$  232 nm; FTIR (CCl<sub>4</sub>) 3555, 3418, 2950, 2930, 2870, 1718, 1650 cm<sup>-1</sup>; <sup>1</sup>H NMR (selected resonances)<sup>26</sup>  $\delta$  1.31 (s, 3 H, C4-CH<sub>3</sub>), 1.43 (s, 3 H, C8-CH<sub>3</sub>), 1.77 (s, 3 H, C2-CH<sub>3</sub>), 2.03 (s, 3 H, C1-CH<sub>3</sub>); <sup>13</sup>C NMR  $\delta$  8.0, 14.0, 19.7, 19.7, 19.8, 22.0, 22.6, 22.7, 23.0, 24.3, 24.9, 25.2, 27.3, 33.1, 33.1, 33.2, 37.7, 37.8, 37.8, 39.8, 41.4, 41.5, 60.3, 78.3, 85.7, 134.1, 168.8, 171.6, 206.9.

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(24) Matsuo, M.; Matsumoto, S.; Iitaka, Y. *J. Org. Chem.* **1987**, *52*, 3514–3520.

(25) Matsuo, M.; Matsumoto, S.; Iitaka, Y. In *The role of Oxygen in Chemistry and Biochemistry*; Ando, W.; Moro-oka, Y., Eds.; Elsevier Science Publishers B.V.: Amsterdam, 1988; pp 503–508.

(26) Proton resonances of the phytol side chain and of the methylene groups in the lactone ring are not reported. Their chemical shift values and integrated areas corresponded to the expected values.