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Novel Tocopherol Compounds VIII. Reaction Mechanism of the Formation of α-Tocored

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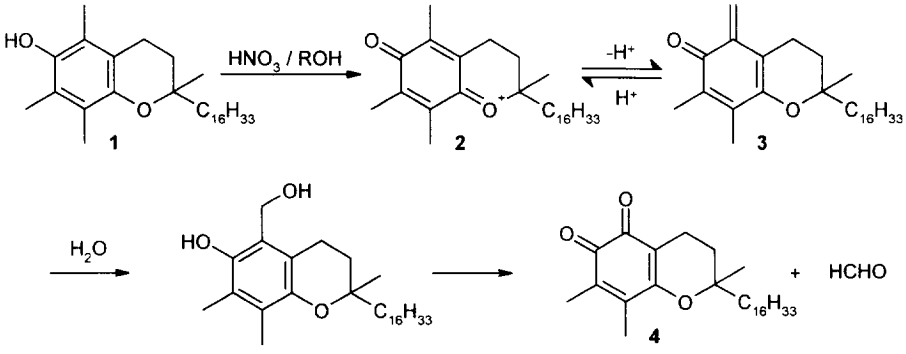
Abstract: A novel mechanism for the formation of 5,6-tocopheryldione (α-tocored, **4**) from α-tocopherol (vitamin E, **1**) has been established. The reaction has been shown to proceed *via* a 5-alkoxytocopherol intermediate, with cleavage of the 5a-methyl group as methanol, contrary to results reported so far in the literature. © 1997 Elsevier Science Ltd. All rights reserved.

The formation of a red colored compound upon oxidation of alcoholic solutions of α-tocopherol (vitamin E, **1**) with diluted HNO_3 or silver nitrate, is one of the oldest reactions in vitamin E chemistry. Discovered by Further and Meyer in 1939,[1] the reaction was applied in an assay for vitamin E for more than two decades, and is still used today to verify the presence of α-tocopherol in biological samples.[2] In 1941, Smith and Ungnade demonstrated that the compound causing the red color was an *ortho*-quinone, 5,6-tocopheryldione (**4**), which they named "α-tocored".[3] They noted that the formation of **4** required the cleavage of the 5a-methyl group, and proposed a mechanism involving the formation of the *ortho*-quinone methide **3** with subsequent addition of H_2O and release of HCHO, see Scheme 1. This proposal was adopted and supported by several working groups so far,[4] and appeared to be widely accepted.



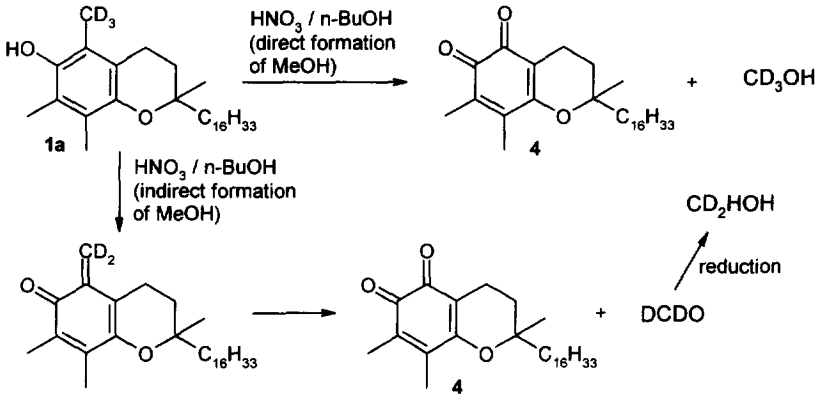
Scheme 1. Previously proposed reaction mechanism[4] for the formation of α-tocored.

Results from our investigations on novel tocopherol derivatives[5] gave rise to doubts about the proposed course of reaction, especially the intermediacy of **3**. Although **3** is a very frequently observed intermediate in vitamin E chemistry, it is preferentially formed by oxidation of α-tocopherol in *aprotic* media, whereas in protic or even aqueous media structures derived from the *para*-quinoid system **2** dominate.[6] Thus, the occurrence of the *ortho*-quinone methide under the conditions used to form α-tocored seemed to be rather problematic. Moreover, all attempts to detect HCHO that should be produced according to the above mechanism failed, but corresponding blank runs with HCHO added together with the α-tocopherol were successful. Therefore, also the cleavage of the 5a-CH_3 group as HCHO during the formation of **4** appeared to be unlikely. Obviously, this 5a-CH_3 group must be removed as C_1-unit in an oxidation stage that theoretically can range from -4 to +4. While the formation of methane (-4) or methanol (-2) were at first believed to be only remote possibilities, the generation of formic acid (+2) or CO_2 (+4) was easier conceivable. To test for these substances we used n-butanol as the solvent for the oxidation and proved that it was inert under the reaction conditions applied.

Surprisingly, GCMS analysis of the product mixture showed the presence of MeOH.[7] The conclusive proof for the generation of methanol was finally provided by the oxidation of α-tocopherol **(1)** to α-tocored **(4)** with HNO_3 at 85°C employing larger amounts of starting material. The evolving gaseous product was condensed in a microdistillation apparatus, dissolved in $CDCl_3$, liberated from water and traces of acid, and determined by NMR spectroscopy. The compound was unambiguously identified as methanol.[8] Thus, as a first result of the experiments, it became clear that the 5a-CH_3 group which is cleaved upon oxidation occurs as *methanol* after the reaction but not as formaldehyde, contrary to assumptions throughout the literature.

There are two possible pathways for the formation of MeOH. First, the 5a-methyl group can be removed directly as methanol by reaction with water. Second, the methyl group would be eliminated as HCHO according to the mechanism assumed so far, but is then reduced to MeOH by NO_x- or tocopherol-based redox systems present in the reaction mixture. In this case, the methanol is formed indirectly. To decide on which pathway is correct we used vitamin E deuterated at position 5a **(1a)**.[9] Formation of α-tocored from this compound would produce CD_3OH if the 5-methyl group is cleaved directly, but CD_2HOH if the "detour" is taken: formation of the *ortho*-quinone methide and release of DCDO which is subsequently reduced to CD_2HOH, see Scheme 2.

When **1a** was oxidized to α-tocored **(4)**, the 5a-methyl group was cleaved as CD_3OH as demonstrated by NMR spectroscopy and MS. This proved - as a second result - that the *ortho*-quinone methide of α-tocopherol **(3)** is *not* an intermediate in the formation of α-tocored, otherwise CD_2HOH would have been formed. The old reaction mechanism illustrated in Scheme 1 is consequently not supported by our experiments. This poses the question of how the *ortho*-quinone **4** is formed, and which intermediates are actually involved.

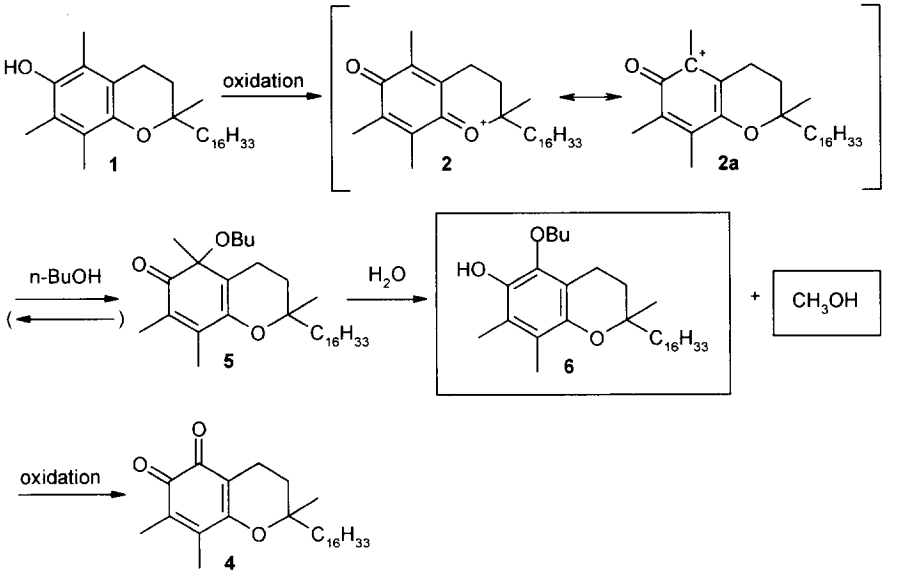


Scheme 2. Formation of α-tocored from 5a-trideutero-α-tocopherol: two possible pathways for the concomitant production of differently deuterated methanol.

It seemed perfectly reasonable that the first step consists in the formation of the *para*-quinoid structure **2**, a reaction typical of the vitamin E oxidation in protic media. To allow cleavage of the 5a-methyl group, a nucleophilic attack at C-5 of the mesomeric *ortho*-quinoid structure **2a** is required as the second step. The resulting cyclohexadienone derivative **5** was expected to be rather labile, and to stabilize itself in subsequent reactions. We were unable to detect **5**, however, we found indeed a quite stable intermediate that was identified as 5-butoxy-γ-tocopherol (**6**), i. e., the starting material with 5a-CH_3 replaced by -OBu.[10] The intermediate was detected by stopping the reaction at different times and analyzing the reaction mixture with GCMS.[11] To make sure that compound **6** was not an artifact of work-up procedure or GC measurements, the mixture was also analyzed without work-up and by the exceptional mild MALDI-MS technique. In all cases, the molecular weight and fragmentation pattern consistent with 5-butoxy-γ-tocopherol (**6**) was detected, a definite proof of its intermediacy in the formation of α-tocored. Through this observation, the reaction mechanism became clear: α-tocopherol is oxidized to the *para*-quinoid structure **2** with the mesomeric form **2a**. This intermediate, in turn, is attacked by the solvent - n-butanol in the present case - at position 5.[12] The resulting cyclohexadienone **5** eliminated the 5a-methyl group as CH_3OH, for instance after protonation of the carbonyl group and nucleophilic attack of water at C-5a. This process actually represents a special case of the well-known acid-promoted cyclohexadienone-phenol rearrangement.[13] The rearomatization after elimination of the 5a-CH_3 group leading to 5-butoxy-γ-tocopherol (**6**) might well be a driving force for the reaction to proceed. Reoxidation of **6** gives the final product, namely 5,6-tocopheryldione (**4**), see Scheme 3.

In summary, the investigation presented was aimed at elucidating the mechanisms of the formation of a frequently occurring oxidation product of vitamin E, α-tocored. A reaction pathway was established and the formation of methanol as the second reaction product was demonstrated. Moreover, 5-alkoxy-γ-tocopherols

were shown to be intermediates of the reaction. In the light of these new results, previously reported mechanistic approaches have to be reevaluated. These findings certainly have an impact on the understanding of the reactivity in the biologically highly important vitamin E system.



Scheme 3. Reaction mechanism for the formation of α-tocored from vitamin E.

EXPERIMENTAL

^{1}H NMR spectra were recorded at 300 MHz, ^{13}C NMR spectra at 75 MHz on a Bruker AC-300P. MALDI-MS experiments were carried out on a Shimadzu time-of-flight instrument with linear geometry (pulsed N_2 laser, 337 nm, pulse duration 3 ns, acceleration voltage 20 kV) with gentisic acid as the matrix. GCMS was performed on a Hewlett Packard (5890 Series II, EI, 70 eV, ITD).

General procedure for the preparation of α-tocored. α-Tocopherol (3.00 mmol, 1.29 g) was mixed with pure n-butanol (80 mL) and 5N HNO_3 (20 mL). The mixture was heated to 60°C for 10 min, and then stirred for 30 min at room temperature. 200 mL of water and 100 mL of n-hexane were added. The organic layer was repeatedly washed with water and dried over Na_2SO_4. Evaporation of the solvent produced pure α-tocored as a red oil with analytical data identical to those reported in the literature.[14] Butanol can as well be replaced for other aliphatic alcohols or acetone in this procedure.

Confirmation of the production of methanol during the formation of α-tocored. In an one-neck flask attached to a microdistillation apparatus, α-tocopherol (20.00 mmol, 8.60 g) was oxidized at 85°C according to the above procedure. The liquid that formed in the condenser was rinsed with $CDCl_3$ and shaken with small

amounts of sodium sulfate and calcium carbonate in a vial. After separation of the solids, the organic layer was analyzed by NMR and GCMS. ^{1}H NMR: δ 3.42 (d, J = 5.1 Hz, 3H, -CH_3), 3.73 (q, 1H, -OH); after addition of CD_3COOD: 4.43 (s, -CH_3). ^{13}C NMR: δ 49.8. MS [m/e (%)]: 32 (70), 31 (100), 29 (75), 15 (40).

A similar experiment was carried out with 2 mmol of 5a-trideutero-α-tocopherol. ^{13}C NMR: δ 48.85 (sept, J = 21.5 Hz, CD_3-). MS [m/e (%)]: 35 (80), 34 (50), 33 (100), 18 (45).

Detection of the intermediate ***6***. A 5 mL sample of the reaction mixture was taken at reaction times between 1 to 10 min at intervals of 1 min, and analyzed by GCMS. In one case, the reaction was stopped after 10 min by neutralization with NaOH. The mixture was extracted with n-hexane. The organic layer was washed with water, dried over Na_2SO_4 and the solvent was evaporated. The obtained mixture containing an excess of α-tocopherol **(1)** besides *para*-tocopheryl quinone, α-tocored **(4)**, and up to 2.5% of 5-butoxy-γ-tocopherol **(6)** was analyzed by GCMS and MALDI-MS. MS [m/e, (%)]: 489 (40, MH^+), 263 (60, M-225 [side chain]), 225 (40, side chain), 223 (80, M-225-41 [propyne]), 167 (30), 166 (80), 57 (100). MALDI-MS: 489 [MH^+]. The data for α-tocopheryl n-butyl ether having a resembling spectrum are given here for the purpose of comparison: MS [m/e, (%)]: 487 (60, MH^+), 261 (10, M-225 [side chain]), 225 (20, side chain), 221 (80, M-225-41 [propyne]), 165 (100), 57 (70).

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REFERENCES AND NOTES

Dedicated to Prof. Dr. K. Schwetlick on the occasion of his 65. birthday

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3. Smith, L. I.; Irwin, W.; Ungnade, H. E. *J. Am. Chem. Soc.* **1939**, *61*, 2424-2429, and following reports.
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5) A series on Novel Tocopherol Compounds was commenced in 1994. For the preceding paper entitled Novel Tocopherol Compounds VII. γ-Tocopherol-5-carboxylic Acid - a Novel Route to γ-Tocopherol see: Rosenau, T., Habicher, W. D. *Synlett* **1997**, *in press.*

6) The formation of the *ortho*-quinone methide in protic media can be forced by application of 5a-substituted tocopherols which eliminate this substituent by treatment with base or oxidants to produce **3**. Rosenau, T., Habicher, W. D. *Tetrahedron* **1995**, *51*, 7919-7926; *J. Org. Chem.* **1995**, *60*, 8120-8121; *Heterocycles*, **1996**, *43*, 787-798.

7) To carry out GC experiments on the reaction mixture is not a trivial task: MeOH must be detected *before* the solvent peak (n-BuOH). Low column pressure and a constant temperature of 130°C worked best. Other C_1 units, such as formic acid or carbon dioxide, were not found.

8) If the reaction is carried out in the presence of $B(OH)_3$, boric acid trimethyl ester $B(OMe)_3$ is obtained instead of methanol.

9) The compound was prepared according to: Hughes, L.; Slaby, M.; Burton, G. W.; Ingold, K. U. *J. Labelled Compd. Radiopharm.* **1990**, *28*, 1049-1057. Ingold, K. U.; Hughes, L.; Slaby, M.; Burton, G. W. *J. Labelled Compd. Radiopharm.* **1987**, *24*, 817-831.

10) When the oxidation is carried out in ethanol, 5-ethoxy-γ-tocopherol was formed as the intermediate. In the absence of aliphatic alcohols, but with water being present, the expected 5-hydroxy-γ-tocopherol could not be detected, due to its lability. That 5-hydroxy-γ-tocopherol is also formed during the oxidation in n-butanol with aqueous HNO_3 cannot be excluded. However, the attack of the excess butanol at C-5 is certainly preferred.

11) The fragmentation patterns of tocopherols and related compounds upon MS or thermal treatment have been thoroughly investigated: Trudell, J. R.; Sample-Woodgate, S. D.; Djerassi, C. *Org. Mass Spectr.* **1970**, *3*, 753-776. Vance, J.; Bentley, R. *Bioorg. Chem.* **1971**, *1*, 345-353. Rosenau, T.; Habicher, W. D. *Heterocycles* **1996**, *43*, 787-798. The MS data obtained on **6** showed unambiguously that methyl (M = 15) at the aromatic ring was replaced by a butoxy group (M = 63). α-Tocopherol butyl ether that has structural features similar to **6**, such as the basic tocopherol backbone and a butoxy group attached to the aromatic ring, exhibits a fragmentation pattern that can easily be distinguished. For data see experimental section.

12) The addition of solvent might be a reversible process, meaning that the formation of the cyclohexadienone **5** from **2a** is an equilibrium. It cannot be excluded that **5** is formed indirectly from **2a**, for example *via* a second intermediate that might involve nitrogen-containing structures derived from HNO_3.

13) *Houben-Weyl*, Müller, E. Ed.; 4th ed., vol. VI/1c; Georg Thieme: Stuttgart, 1976; pp. 735-799.

14) For analytical data of **4** see: references 1, 3, 4 and Brownstein, S.; Burton, G. W.; Hughes, L.; Ingold, K. U. *J. Org. Chem.* **1989**, *54*, 560-569.

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