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Novel tocopheryl compounds XXIV. Studies into the nitrosation chemistry of γ-tocopherol: preparation of 5-nitroso-γ-tocopherol via an organomercury derivative of vitamin E

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Abstract—5-Nitroso-γ-tocopherol (**5**) was not accessible by direct nitrosation of γ-tocopherol (**3**), but was synthesized for the first time by nitrosation of an organomercurial intermediate (**11**) under aprotic conditions. Under protic conditions **5** exists in equilibrium with its *ortho*-quinone monoxime tautomer **6**, the latter being the major component with >99%. NMR and analytical data of the tautomeric couple are reported for the first time. The chemistry of the nitrosation of γ-tocopherol was studied in detail. In the presence of oxygen, **5** is readily oxidized to 5-nitro-γ-tocopherol (**7**), whereas at elevated temperatures an additional process, the conversion of monoxime **6** into 5,6-tocopheryldione (**8**) under loss of hydroxylamine sets in. The experimental results agreed with the outcome of DFT computations.

1. Introduction

Vitamin E, usually taken as a term to describe α-tocopherol or even its acetate, is actually a mixture of four tocopherols (**1**–**4**) and four tocotrienols.[1,2] The tocopherols, distinguished by the Greek prefixes α to δ, differ in the number and position of methyl groups at the aromatic ring. All tocopherols are good antioxidants, the α-form having the highest vitamin E activity.[3,4] In contrast to α-tocopherol (**1**) where all aromatic positions are substituted, the non-α-tocopherols (**2**–**4**) possess free aromatic positions and are thus susceptible to electrophilic aromatic substitution. This reaction type had been studied mainly for γ-tocopherol (**3**), and meanwhile it has become well established that the γ-isomer in contrast to the α-isomer is an efficient trap of electrophiles under physiological conditions, whereas the antioxidant activity, i.e., radical scavenging ability, of the α-isomer is the higher one.

In a current project we are studying reactivity differences of non-α-tocopherols toward electrophiles or electrophile precursors, such as peroxynitrite, hypohalite, or thiocyanate, produced by the action of peroxidases or myeloperoxidases in different systems. In this paper, we would like to clarify the reactions occurring upon nitrosation of γ-tocopherol and present the first synthesis of 5-nitroso-γ-tocopherol (**5**) and its *ortho*-quinone monoxime tautomer (**6**). Due to the general dominance of the latter, a 'detour' avoiding protic conditions was required to synthesize the nitroso compound, which involved preparation of the first organomercurial derivative of vitamin E.

2. Results and discussion

5-Nitro-γ-tocopherol (**7**) has been described as nitration product of γ-tocopherol (**3**), but also as the product of the reaction between γ-tocopherol and nitrosating species, such as NO or nitrous acid.[5–11] Astonishingly, it has rarely been regarded worth mentioning that actually 5-nitroso-γ-tocopherol (**5**) should be formed as the initial product of nitrosation, rather than the nitro compound **7**. In most studies involving those nitrosating and nitrating reaction systems, conversions under physiological or near-physiological conditions were monitored, and the course of the reaction was followed by UV. The fact that different product UV spectra were obtained was attributed to changing ratios between 5-nitroso product and 5-nitro product and to the oxidation of the former to the latter by contact with oxygen,[12] which appeared to be a reasonable explanation at the first glance. However, it was a bit surprising—especially with regard to the physiological and medical importance of the system—that in no case analytical data of 5-nitroso-γ-tocopherol, apart from UV spectra, had been presented so far.

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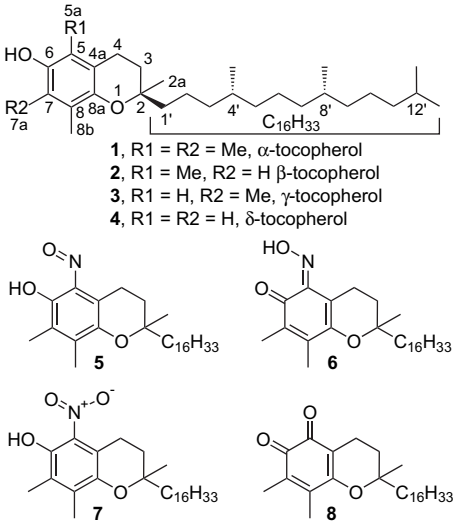
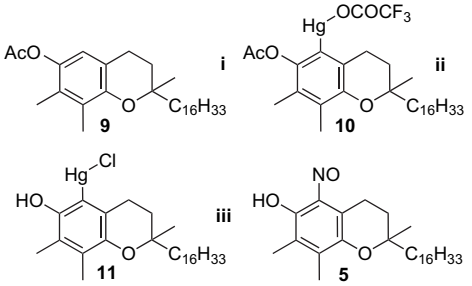


To obtain an authentic sample of 5-nitroso-γ-tocopherol (**5**) we performed nitrosation of γ-tocopherol under various different conditions, e.g., involving NO, NO_2 or peroxynitrite in enzyme-based systems, but failed completely to obtain the nitroso product. Additionally, inorganic nitrites with and without mineral acids as well as organic nitrites were tested, but with the same negative outcome. Mixtures of three to four main components were produced, from which isolation and identification of two products readily succeeded, the nitro derivative **7** and *ortho*-quinone **8** (5,6-tocopheryldione, α-tocored). Alternative approaches, nitrosation with nitrosyl tetrafluoroborate or nitrosyl chloride, were performed under various conditions. But also this reaction type, known to afford quinoid side products,[5] produced **7** and **8** in fair to good yields, but failed to afford the desired 5-nitroso derivative.

The oxidation of the nitroso into the corresponding nitro derivative was unlikely to account for the complete lack of nitrosation product: this process, even though well known to proceed readily, is not an immediate, but a rather gradual process.[12] It was more likely instead that the well-known tautomerism between *ortho*-nitrosophenols and the corresponding *ortho*-quinone monoximes[13–15] played a crucial role here. This tautomerism and its energetics have been a prime target for theoretical and spectroscopic studies: in the case of the non-substituted parent compound nitrosophenol, the nitroso form was established to be slightly energetically preferred over the oxime form, whereas for naphthol and phenanthrene derivatives the situation was reversed and the oxime tautomer was more stable.[13–15]

In order to obtain 5-nitroso-γ-tocopherol (**5**) in reasonably pure form as a reference compound, we also attempted nitrosation of *O*-protected derivatives of γ-tocopherol followed by O-deprotection. The synthesis of the 5-nitroso derivatives of γ-tocopheryl acetate, γ-tocopheryl methyl ether, and *O*-trimethylsilyl γ-tocopherol succeeded,[16] but unfortunately all deprotection attempts to liberate the phenolic hydroxyl group and to obtain the free nitrosophenol failed. Evidently, aqueous and especially acidic conditions immediately established the equilibrium between nitrosophenol and *ortho*-quinone monoxime tautomer, which was quite far on the side of the latter. Thus, we were forced to avoid protic conditions in the preparation of the nitroso derivative. This limitation and the fact that all direct nitrosation attempts had failed in our hands—or, more correctly, had provided mixtures containing the oxime tautomer instead of the desired nitroso compound—left us with a rather limited range of synthetic options. Thus, a detour was taken, involving the preparation of a organomercury derivative of γ-tocopherol, which in a second step was directly converted into the nitrosophenol in aprotic media (Scheme 1).



i = $Hg(OCOCF_3)$, 0 °C, 45 min; **ii** = HCl, reflux, 10 min. 82%
iii = $NOBF_4$, Et_2O to *n*-hexane, 2 h, 78%

Scheme 1. Synthesis pathway toward 5-nitroso-γ-tocopherol (**5**).

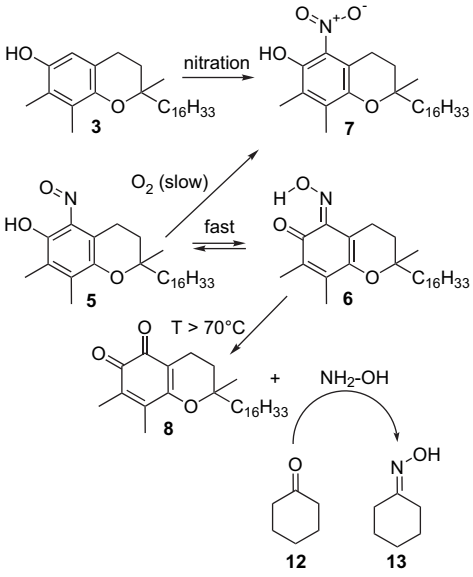
To avoid the well-known side reactions of the free phenolic OH group during mercurization, γ-tocopheryl acetate (**9**) was used as the starting material. The strongly electrophilic mercury(II) trifluoroacetate gave complete conversion to trifluoroacetomercurial derivative **10** and a neat reaction even at 0 °C and was thus superior to both mercury(II) chloride and mercury(II) acetate, which required refluxing ethanol for complete conversion and produced tocopherol-derived by-products. The use of γ-tocopheryl acetate in slight excess relative to the mercury salt offered some advantages in work-up over the opposite approach with the inorganic salt being used in excess. The non-reacted tocopherol was readily separated by column chromatography whereas excess mercury salt was rather inconvenient to separate completely, and interfered with the quality of the chromatographic separation. The refluxing step, following the actual mercurization reaction without isolation of intermediate **10**, served two purposes: through the concentrated HCl the *O*-acetate is cleaved under liberation of the phenolic group, and the organomercurial is converted into the more stable and less soluble chloromercury form. The deprotection of the phenolic hydroxyl was necessary at this stage, i.e., before nitrosation. Deprotection after the nitrosation step would inevitably cause the benzoquinone monoxime tautomer of nitroso-γ-tocopherol to be formed as discussed above, and was thus no option. 5-Chloromercurio-γ-tocopherol (**11**) represents the first organomercurial derivative of tocopherols. In pure form, it is an ivory wax with mother-of-pearl appearance, solidifying in rosette-like, radial patterns. The compound is little soluble in cold *n*-hexane, soluble in warm glacial acetic acid, and dissolves in acetonitrile with green fluorescence. At 40 °C it becomes pasty but does not melt, at temperatures above 95 °C decomposition under release of mercury sets in. Under ambient conditions under nitrogen and at −20 °C under air it was stable. Characteristic NMR features of **11**, in agreement with data of other organomercurials,[17,18] are the large value of the $^1J_{C-Hg}$ coupling constant with more than 2200 Hz, and vicinal mercury–carbon couplings ($^3J_{C-Hg}$) being larger than the geminal ones ($^2J_{C-Hg}$).

5-Chloromercurio-γ-tocopherol (**11**) was directly converted into 5-nitroso-γ-tocopherol (**5**). While the conversion of aryl organomercurials into nitroso compounds involving protic conditions has been known for long,[19,20] only a recently published procedure opened the way to conduct the synthesis under strictly aprotic conditions,[21] which was adopted for our purpose and yield-optimized. According to the final procedure, nitrosyl tetrafluoroborate was used for nitrosation, which proved to be superior to *tert*-butylnitrite and far better than nitrosyl chloride or isoamylnitrite. Diethyl ether was a good solvent for the nitrosation to proceed in near-quantitative yields. However, the water content of the ether was a crucial factor. Already traces of moisture—in combination with the nitrosonium salts used—caused trace formation of acid, which in turn triggered the formation of the quinone oxime tautomer—readily visible by the dark red color of the solution. Inorganic carbonate was added to reduce the danger of local acidic reaction. Under strictly aprotic conditions, the reaction mixture maintained a yellow color throughout. Exchange of the diethyl ether to *n*-hexane in combination with solid KCl as flocculation aid allowed separation of the mercury salts. The obtained yellow solution contained 5-nitroso-γ-tocopherol (**5**) as the main component (approx. 82%) besides three by-products: 5-nitro-γ-tocopherol (**7**, approx. 10%) as well as *ortho*-quinone **8** and the *para*-quinone of γ-tocopherol (each less than 5%).

Isolation of 5-nitroso-γ-tocopherol (**5**) in substance proved to be impossible. While concentration of solutions at rt or below was possible, any attempt to remove the solvent completely caused red discoloration indicating the conversion into monoxime (**6**). Similarly, the compound was immediately converted into its tautomer upon contact to silica gel, alumina (both neutral and basic), and any protic media. For NMR measurements (Table 1), the solvent DMSO-d_6 was thus added to the *n*-hexane solution, and the alkane was removed subsequently by evaporation. $CDCl_3$ was inappropriate due to possible acidic reactions and the small boiling point difference was relative to *n*-hexane. NMR samples were kept under an atmosphere of ammonia. In general, during preparation and analysis caution must be exercised to avoid local acidic reactions as well as complete solvent removal since both processes cause immediate conversion of **5** into its more stable quinone monoxime tautomer **6**.

The synthetic approach according to Scheme 1 allowed us synthesizing the bright yellow 5-nitroso-γ-tocopherol (**5**) to obtain reliable NMR and MS data, but only as solution. The oxime tautomer **6**, however, was readily isolable and was refined to analytical purity by preparative column chromatography in apolar media. However, it should be emphasized again that this red compound—although having the same molecular formula as **5**—is not the actual nitrosation product of γ-tocopherol, but its more stable quinoid tautomer. In this regard it is necessary to note that all literature accounts having reported a red color of the nitrosation product or UV bands typical of quinones had actually detected the tautomeric mixture with more than 99% quinoid **6**, rather than the pure 5-nitroso-γ-tocopherol (**5**) itself.

The nitrosophenol–quinone oxime tautomerism governing the chemistry of the system under study was studied in more detail (Scheme 2). 5-Nitroso-γ-tocopherol (**5**) formed bright yellow solutions in diethyl ether and *n*-hexane. Its tautomer, the 5-*O*-oxime of 5,6-*ortho*-tocopherylquinone (**6**)—actually the product of a [1,6]-sigmatropic proton shift—was a red wax imparting an intense ruby red color on its organic solutions. Upon contact of **5** with protic solvents, such as ethanol, the formation of the tautomer proceeded within a few seconds, in the presence of acids it became an immediate process. When heated above 70 °C



Scheme 2. Nitrosation chemistry of γ-tocopherol: tautomeric equilibrium between 5-nitroso-γ-tocopherol (**5**) and 5,6-tocopheryldione 5-oxime (**6**) and further reactions to 5-nitro-γtocopherol (**7**) by air oxidation of **5** and formation of α-tocored (**8**) from **6** by loss of hydroxylamine accelerated by NH_2OH traps.

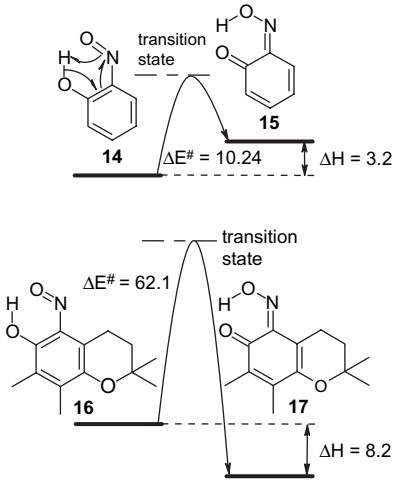
Table 1. Characteristic NMR resonances to distinguish the major components in nitrosation/nitration systems of γ-tocopherol (all values in ppm, ^{13}C resonances rounded to the first decimal, $CDCl_3$, standard TMS)

Nuclei	5-Nitroso-γ-tocopherol (**5**)[a]	Monoxime tautomer **6**	5-Nitro-γ-tocopherol (**7**)	*ortho*-Quinone **8**
C-5	160.3	144.9	134.2	177.8
C-6	134.2	181.9	148.0	180.8
C-4a	120.1	110.0	113.1	110.0
C-7	124.0	127.6	125.4	134.1
C-8	136.1	147.1	137.1	143.6
C-8a	146.4	142.6	144.0	163.2
H-4	2.97 (t)	2.229 (t)	2.96 (t)	2.41 (t)
H-7a/H-8b	2.12 (s)/2.15 (s)	1.95 (s)/2.00 (s)	2.11 (s)/2.14 (s)	1.93 (s)/2.00 (s)

[a] DMSO-d_6 (see text).

in protic solvents, the monoxime tautomer **6** slowly released hydroxylamine,[22] being converted at the same time into the free *ortho*-quinone **8**. The release of hydroxylamine was accelerated by flushing with a stream of argon, in which hydroxylamine was confirmed by GC–MS. Addition of cyclohexanone (**12**) to the nitrosation product dissolved in acidified (TFA) refluxing ethanol even allowed to quantitatively convert the nitroso/oxime couple into α-tocored (**8**) within less than 1 h. The quinone monoxime component was constantly consumed and the released hydroxylamine directly converted into the stable cyclohexanone oxime (**13**), see Scheme 2. Cyclohexanone was chosen as trapping agent because of the stability of the oxime trapping product **13**, its well-known chemistry and behavior in GC–MS analysis—cyclohexanone oxime is the starting material for the production of ε-caprolactame—and the lack of cis/trans-oxime isomers due to the symmetric shape of the ketone.

The conclusion drawn from synthesis and NMR spectroscopy (Table 1) was that the tautomeric equilibrium between nitroso and quinone form was far on the side of the latter, which is in agreement with computations on the couple **16/17**—as a truncated model of the pair **5/6**—at the B3LYP/6-311+G(2df,2p) level of theory. We used this theoretical level as it was applied previously to study the model system *o*-nitrosophenol (**14**)/*o*-benzoquinone monoxime (**15**), see Scheme 3. The energy difference calculated there was 3.2 kcal mol^{-1} in favor of the nitroso tautomer.[13] Our computations on the couple **16/17** afforded a value of 8.2 kcal mol^{-1} in favor of the oxime.[23] With 62.1 kcal mol^{-1} the barrier of tautomerization from **16** to **17** (and thus from **5** to **6**) was higher than the value calculated previously for the model system **14/15** (10.24 kcal mol^{-1}).[14] The higher value is understandable as the ring strain effect of the annulated pyran ring is different for aromatic and quinoid structures, according to the SIBL principle (strain-induced bond localization) as recently studied for vitamin E-type compounds.[24,25] Both calculated values, the energy difference and the activation energy, agreed with the experimental facts,



Scheme 3. Calculated relative energies and tautomerization barriers (B3LYP/6-311+G(2df,2p)) in the model system *o*-nitrosophenol (**14**)/*o*-benzoquinone monoxime (**15**) (data from Refs. 13,14) and in the analogous system **16/17** with a substitution pattern typical of tocopherols. Compounds **16** and **17** were used for calculation as truncated (CH_3 instead of isoprenoid side chain) model compounds of the tautomeric couple 5-nitroso-γ-tocopherol (**5**)/5,6-tocopheryldione 5-oxime (**6**). All values in kcal mol^{-1}.

namely the favorization of the oxime under equilibrium conditions and the possibility to isolate the nitroso form under aprotic conditions disfavoring tautomerization. The typical substituent pattern of tocopherols present in **5/6** and in the computational models **16/17** evidently reversed the stability order of the tautomers as compared to the non-substituted couple **14/15**.

3. Conclusion

Nitrosation of γ-tocopherol (**3**) produces 5-nitroso-γ-tocopherol (**5**), which in protic media is immediately transformed into its tautomer, 5,6-tocopheryldione 5-oxime (**6**). In protic media and thus also under physiological conditions, the quinoid tautomer **6** largely dominates, the equilibrium concentration of the nitroso form being below 1%. Synthesis of the nitroso form **5** succeeded under non-protic conditions via 5-chloromercurio-γ-tocopherol (**11**), the first organomercury derivative of the vitamin E compound class. DFT computations supported the experimentally observed preference of the oxime tautomer and afforded a tautomerization barrier of 62 kcal mol^{-1}.

In the presence of oxygen, the tautomeric couple was slowly oxidized to 5-nitro-γ-tocopherol (**7**). At elevated temperatures, the monoxime tautomer became unstable and was converted into *ortho*-quinone α-tocored (**8**) by release of hydroxylamine. The final, stable product found in nitrosation mixtures of γ-tocopherol thus depends strongly on the conditions: 5-nitro-γ-tocopherol (**7**) at ambient temperatures in the presence of oxygen, and monoxime **6** (with less than 1% of nitroso tautomer **5** present) at ambient temperature in the absence of oxygen. At temperatures above 70 °C, a mixture of nitro derivative **7** and *ortho*-quinone **8** is obtained under oxidative conditions, and solely *ortho*-quinone **8** in inert atmosphere.

Experiments involving nitrosation/nitration chemistry of γ-tocopherol and corresponding analytical approaches have to consider the complexity of the system. Nitrosation reactions under physiological conditions will not afford a pure product, but a mixture of at least three components—the *ortho*-quinone monoxime **6** as major product with some amounts of the nitroso product **5** as minor tautomeric component and the 5-nitro derivative **7**. With increasing reaction time the composition of the mixtures will change as the amount of **7** is increased by oxidation while the two tautomers are consumed, their ratio remaining constant, as it is set by the equilibrium. At elevated temperatures, even another product (α-tocored **8**) comes into play. The UV spectra of such complex reaction mixtures will inevitably be largely different and intricate—as confirmed by the diversity of literature reports—and naturally cannot be assigned to one specific compound as hitherto attempted. The UV data are only valuable in combination with the defined reaction conditions and reaction time, which determine the composition at the time of measurement.

The present work is seen as an attempt to clarify the nitrosation/nitration chemistry of non-α-tocopherols, which is highly relevant with regard to the physiological and medicinal importance of these compounds as traps of NOx electrophiles in mammalian tissues.

4. Experimental

4.1. General

All chemicals were commercially available and used without further purification. Reagent-grade solvents were used for all extractions and workup procedures. Distilled water was used for all aqueous extractions and for all aqueous solutions. *n*-Hexane, diethyl ether, ethyl acetate, and petroleum ether used in chromatography were distilled before use. γ-Tocopherol was of the [R,R,R]-type, maintenance of stereochemical integrity over the reactions performed was not checked, however.

All reactions involving non-aqueous conditions were conducted in oven-dried (140 °C, overnight) or flame-dried glassware under an argon or nitrogen atmosphere. TLC was performed using Merck silica get 60 F_{254} pre-coated plates. Flash chromatography was performed on Baker silica gel (40 μm particle size). All products were purified to homogeneity by TLC–GC analysis; yields refer to isolated, pure products with satisfying elemental analysis data. Melting points, determined on a Kofler-type micro hot stage with Reichert-Biovar microscope, are uncorrected. ^{1}H NMR spectra were recorded at 300.13 MHz for ^{1}H and at 75.47 MHz for ^{13}C NMR in $CDCl_3$ if not otherwise stated. Chemical shifts, relative to TMS as internal standard, are given in δ values and coupling constants in hertz. ^{13}C peaks were assigned by means of APT, HMQC, and HMBC spectra. The nomenclature of tocopherols and chromanols as recommended by IUPAC[26,27] was used throughout. Resonances of the isoprenoid side chain of tocopherols are not influenced by modifications of the chroman ring,[28,29] but are listed nevertheless for the purpose of comprehensiveness. 'd.i.' denotes peaks with double intensity. Elemental analyses were performed at the Microanalytical Laboratory of the Institute of Physical Chemistry at the University of Vienna.

4.2. Computations

Computations, as implemented through Spartan Pro 02 by Wavefunction, Inc., Irvine, CA, USA, were carried out on geometries pre-optimized by the semi-empirical PM3 method. For full geometry optimization the widely employed B3LYP hybrid method, which includes a mixture of HF and DFT exchange terms and the gradient-corrected correlation functional of Lee et al.[30,31] parameterized by Becke,[32,33] was used, along with the double-zeta split valence basis sets 6-31+G*,[34,35] which includes diffuse functions, or the higher 3-311-G(2df,2p) analogue. Vibrational frequencies were calculated at the respective level of theory to characterize local minima (equilibrium structures) or first-order saddle points (transition states) on the potential energy surface and to determine zero-point vibrational energies. All equilibrium geometries were characterized by real frequencies only, all transition states by one imaginary frequency.

4.3. Studies into the nitrosation of γ-tocopherol

4.3.1. 5-Chloromercurio-γ-tocopherol (11). In an argon atmosphere at 0 °C, an ethanolic solution of mercury(II) trifluoroacetate (426 mg, 1.0 mmol) was added to a solution of γ-tocopheryl acetate (500 mg, 1.1 mmol) in absolute ethanol. The mixture was stirred at 0 °C until TLC control indicated complete conversion (approx. 45 min), and stirring was continued at rt for an additional hour. Concentrated HCl (1 mL) was added and the mixture was refluxed for 10 min. The solvent was evaporated in vacuo, and the oily residue was chromatographed on silica gel (gradient from *n*-hexane to *n*-hexane/ethyl acetate, v/v=5:1) to provide **11** as an ivory wax (535 mg, 82%). ^{1}H NMR: δ 1.70 (t, 2H, ^3J=6.9 Hz, H-3), 2.08 (s, 3H, H-8b), 2.13 (s, 3H, H-7a), 2.72 (t, 2H, ^3J=6.9 Hz, H-4). ^{13}C NMR: δ 12.23 (C-7a), 13.19 (C-8b), 21.88 (C-4, $^3J_{C-Hg}$=33.8 Hz), 26.25 (C-2a), 31.93 (C-3), 73.86 (C-2), 115.21 (C-4a, $^2J_{C-Hg}$=96.5 Hz), 122.18 (C-7, $^3J_{C-Hg}$=186.3 Hz), 129.13 (C-8, $^4J_{C-Hg}$=36.5 Hz), 142.88 (C-5, $^1J_{C-Hg}$=2295 Hz), 143.32 (C-8a, $^3J_{C-Hg}$=177.8 Hz), 148.12 (C-6, $^2J_{C-Hg}$=103.0 Hz); isoprenoid side chain: 19.66 (C-4a′), 19.75 (C-8a′), 21.20 (C-2′), 22.61 (C-13′), 22.72 (C-12a′), 24.47 (C-6′), 24.77 (C-10′), 27.90 (C-12′), 32.62 (C-8′), 32.69 (C-4′), 37.25 (C-7′), 37.38 (C-9′), 37.50 (C-5′), 37.52 (C-3′), 39.31 (C-11′), 40.04 (C-1′). M=651.73. Anal. Calcd for $C_{28}H_{47}ClHgO_2$: C, 51.60; H, 7.27; Cl, 5.44; Hg, 30.78. Found: C, 51.52; H, 7.22; Cl, 5.64; Hg, 30.92 (gravimetric).

4.3.2. 5-Nitroso-γ-tocopherol (5). At 0 °C in an argon atmosphere, a solution of nitrosyl tetrafluoroborate (12 mg, 1.025 mmol) in dry ethyl ether (3 mL) was added to a solution of 5-chloromercurio-γ-tocopherol (**11**, 65 mg, 0.1 mmol) in dry ethyl ether (2 mL) containing anhydrous potassium carbonate (0.05 g). The mixture was stirred for 2 h, *n*-hexane (20 mL) and dry potassium chloride (0.1 g) were added to completely precipitate the mercury salts. After stirring for 5 min at rt the solids were separated by filtration. DMSO-d_6 was added and the hexane was removed in vacuo at temperatures not exceeding rt. The yellow solution contained 5-nitroso-γ-tocopherol (**5**) as the main component (approx. 82%, residual by-products are mentioned in the main text). The sample was kept under an atmosphere of ammonia and was used directly for NMR measurements. ^{1}H NMR (DMSO-d_6): δ 1.69 (t, 2H, ^3J=6.7 Hz, H-3), 2.12 (s, 3H, H-7a/8b), 2.15 (s, 3H, H-7a/8b), 2.97 (t, 2H, ^3J=6.7 Hz, H-4). ^{13}C NMR: δ 11.98 (C-7a), 13.14 (C-8b), 21.44 (C-4), 26.85 (C-2a), 32.34 (C-3), 73.29 (C-2), 120.13 (C-4a), 123.96 (C-7), 134.22 (C-6), 136.12 (C-8), 146.36 (C-8a), 160.34 (C-5); isoprenoid side chain: 19.72 (C-4a′), 19.83 (C-8a′), 21.25 (C-2′), 22.75 (C-13′), 22.78 (C-12a′), 24.60 (C-6′), 24.77 (C-10′), 28.02 (C-12′), 32.67 (C-8′), 32.79 (C-4′), 37.35 (C-7′), 37.50 (C-9′), 37.54 (C-5′), 37.38 (C-3′), 39.35 (C-11′), 39.70 (C-1′). HRMS (ESI Q-TOF) *m/z*: calcd for $C_{28}H_{47}NO_3$: $[MH]^+$ 446.6997, found $[MH]^+$ 446.7003.

4.3.3. 5,6-Tocopheryldione 5-oxime (6). The above procedure for the synthesis of **5** was repeated starting from 200 mg (0.31 mmol) of 5-chloromercurio-γ-tocopherol (**11**). Into the yellow solution in *n*-hexane obtained two drops of TFA was added. The color changed immediately to ruby red. The solution was passed through layers of dry potassium carbonate and Celite, the solvents were evaporated, and the residue was chromatographed on silica gel (*n*-hexane) to afford **6** as a red viscous oil (78%, 107 mg), elution order was **8**, **6**, and **7**. ^{1}H NMR: δ 1.72 (m, 2H, H-3), 1.95 (s, 3H, H-8b), 2.00 (s, 3H, H-7a), 2.29 (t, 2H, ^3J=6.6 Hz, H-4). ^{13}C NMR: δ 11.53 (C-7a), 13.32 (C-8b), 16.22 (C-4), 23.72 (C-2a), 32.21 (C-3), 81.55 (C-2), 109.98 (C-4a), 127.65 (C-7),

147.14 (C-8), 142.56 (C-8a), 144.88 (C-NOH), 181.87 (CO); isoprenoid side chain: 19.62 (C-4a′), 19.71 (C-8a′), 21.12 (C-2′), 22.61 (C-13′), 22.70 (C-12a′), 24.51 (C-6′), 24.75 (C-10′), 27.99 (C-12′), 32.60 (C-8′), 32.72 (C-4′), 37.29 (C-7′), 37.40 (C-9′), 37.52 (C-5′), 37.55 (C-3′), 39.33 (C-11′), 39.93 (C-1′). Anal. Calcd for $C_{28}H_{47}NO_3$: C, 75.46; H, 10.63; N, 3.14. Found: C, 75.52; H, 10.68; N, 3.17.

5-Nitro-γ-tocopherol (**7**) as well as the *ortho*-quinone α-tocored (**8**) are side products in the nitrosation chemistry of γ-tocopherol. The analytical data reported in the literature were not consistent for both tocored[36–39] and the nitro derivative.[5–7,9,10] In the case of the former many reports date before the 1960s and include UV spectra, but no NMR data nor proof of purity, in the case of the latter conclusions on the nature of the compound were often based solely on UV spectra and were thus—due to the equilibria and side reactions described above—quite erroneous. Thus, we would like to provide full NMR and analytical data for the reason of comparison and comprehensiveness.

4.3.4. 5-Nitro-γ-tocopherol (7). Red oil, TLC: R_f=0.65 (*n*-hexane/diethyl ether, v/v=9:1). ^{1}H NMR: δ 1.67 (t, 2H, ^3J=6.9 Hz, H-3), 2.11 (s, 3H, H-7a/8b), 2.14 (s, 3H, H-7a/8b), 2.96 (t, 2H, ^3J=6.8 Hz, H-4), 10.65 (s, 1H, –OH). ^{13}C NMR: δ 11.87 (C-7a), 13.05 (C-8b), 21.83 (C-4), 23.99 (C-2a), 31.14 (C-3), 75.02 (C-2), 113.12 (C-4a), 125.43 (C-7), 134.22 (C-5), 137.12 (C-8), 144.03 (C-8a), 148.00 (C-6); isoprenoid side chain: 19.76 (C-4a′), 19.84 (C-8a′), 21.09 (C-2′), 22.67 (C-13′), 22.74 (C-12a′), 24.50 (C-6′), 24.65 (C-10′), 28.00 (C-12′), 32.64 (C-8′), 32.75 (C-4′), 37.33 (C-7′), 37.46 (C-5′), 37.47 (C-9′), 37.52 (C-3′), 39.32 (C-11′), 39.69 (C-1′). Anal. Calcd for $C_{28}H_{47}O_4N$: C, 72.84; H, 10.26; N, 3.03. Found: C, 72.78; H, 10.42; N, 2.94.

4.3.5. 5,6-Tocopheryldione (α-tocored, 8). Red wax, TLC: R_f=0.55 (*n*-hexane). ^{1}H NMR: δ 1.72 (m, 2H, H-3), 1.93 (s, 3H, H-8b), 2.00 (s, 3H, H-7a), 2.41 (t, 2H, ^3J=6.6 Hz, H-4). ^{13}C NMR: δ 11.45 (C-7a), 13.61 (C-8b), 15.33 (C-4), 23.75 (C-2a), 32.48 (C-3), 81.22 (C-2), 110.04 (C-4a), 134.06 (C-7), 143.62 (C-8), 163.22 (C-8a), 177.78 (C-5); 180.78 (C-6); isoprenoid side chain: 19.62 (C-4a′), 19.71 (C-8a′), 21.12 (C-2′), 22.61 (C-13′), 22.70 (C-12a′), 24.51 (C-6′), 24.75 (C-10′), 27.99 (C-12′), 32.60 (C-8′), 32.72 (C-4′), 37.29 (C-7′), 37.40 (C-9′), 37.52 (C-5′), 37.55 (C-3′), 39.33 (C-11′), 39.93 (C-1′). Anal. Calcd for $C_{28}H_{46}O_3$: C, 78.09; H, 10.77. Found: C, 78.00; H, 11.02.

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