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Vitamin E Chemistry. Nitration of Non-α-tocopherols: Products and Mechanistic Considerations

Anjan Patel,[†] Falk Liebner,[†] Thomas Netscher,[‡] Kurt Mereiter,[§] and Thomas Rosenau*[,†]

Department of Chemistry, University of Natural Resources and Applied Life Sciences, Muthgasse 18, A-1190 Vienna, Austria, Research and Development, DSM Nutritional Products, P.O. Box 3255, CH-4002 Basel, Switzerland, and Department of Chemistry, Vienna University of Technology, Getreidemarkt 9, A-1060 Vienna, Austria

thomas.rosenau@boku.ac.at

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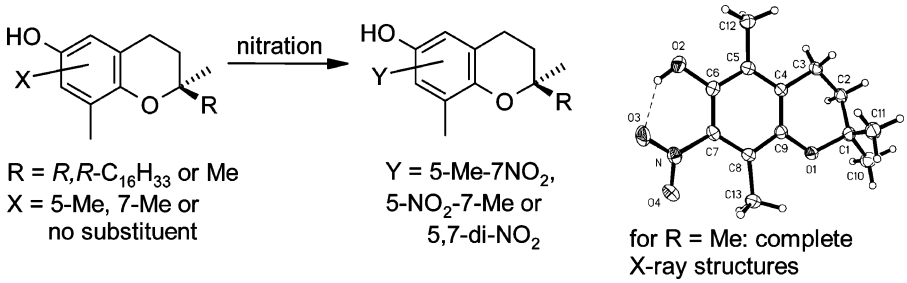

In contrast to the α-form permethylated at the aromatic ring, non-α-tocopherols possess free aromatic ring positions which enable them to act as potent scavengers of electrophiles in vivo and in vitro. In preparation of enzymatic studies involving peroxynitrite and other nitrating systems, the behavior of non-α-tocopherols under nitration conditions was studied. The nitration products of *β*-, *γ*-, and *δ*-tocopherol were identified, comprehensively analytically characterized, and their structure was supported by X-ray crystal structure analysis on truncated model compounds. Even under more drastic nitration conditions, no erosion of the stereochemistry at 2-C occurred. The nitrosation of *γ*-tocopherol and *δ*-tocopherol was re-examined, showing the slow oxidation of the initial nitroso products to the corresponding nitro derivatives by air to be superimposed by a fast equilibrium with the tautomeric *ortho*-quinone monoxime, which only in the case of *γ*-tocopherol released hydroxyl amine at elevated temperatures to afford the stable *ortho*-quinone. Mononitration of *δ*-tocopherol selectively proceeded at position 5. This selectivity can be explained by the theory of strain-induced bond localization (SIBL) to the quinoid nitration intermediates. Bisnitration was only insignificantly disfavored by the first nitro group, so that under normal nitration conditions offering an excess of nitrating species only the bisnitration product was found.

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] The naturally occurring tocopherols **1**−**4** are single-isomer compounds having (2*R*,4′*R*,8′*R*) stereochemistry.

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 more comprehensively when a possible role of *γ*-tocopherol as a trap of electrophiles, especially NO, shifted into the focus of interest. It is now established that the *γ*-homologue—in contrast to the

[†] University of Natural Resources and Applied Life Sciences.
[‡] DSM Nutritional Products
[§] Vienna University of Technology.

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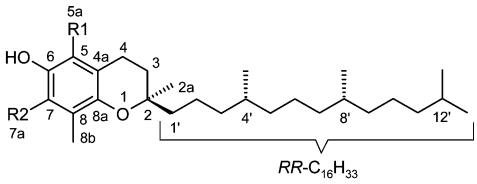
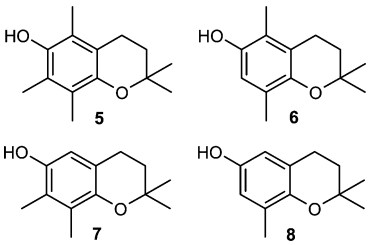
α-homologue[5,6]—is well able to trap electrophiles under physiological conditions, and it has been detected in human plasma and tissue samples.[7−9] Analogous studies of the β- and δ-homologues do not exist.



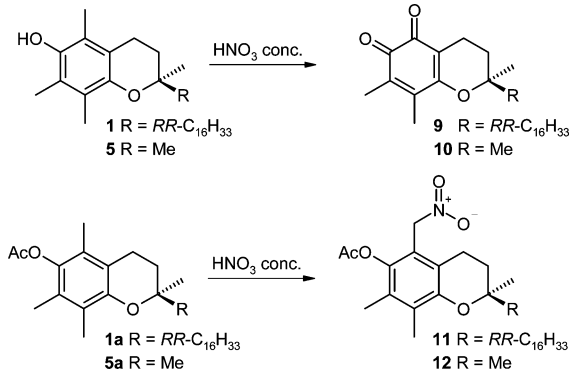
1, R1 = R2 = Me, *(2R,4′R,8′R)*-α-tocopherol
2, R1 = Me, R2 = H, *(2R,4′R,8′R)*-β-tocopherol
3, R1 = H, R2 = Me, *(2R,4′R,8′R)*-γ-tocopherol
4, R1 = R2 = H, *(2R,4′R,8′R)*-δ-tocopherol



α-Tocopherol (**1**) and its model compound 2,2,5,7,8-pentamethylchroman-6-ol (**5**, PMC) have no free aromatic ring positions and thus cannot be nitrated or otherwise electrophilically substituted. In earlier work, we have shown by isotopic labeling studies that the nitration of α-tocopherol under rather drastic conditions causes formation of an *ortho*-quinone, α-tocored (**9**), or its short-chain analogue **10**, according to a multistep mechanism consisting of a [1,3]-sigmatropic shift of the 5a-methyl group from 5-C to 6-O with subsequent further oxidation to the *ortho*-quinone under release of the 5a-C moiety as methanol.[10] α-Tocopheryl acetate (**1a**) and its model **5a**—for which a reaction under nitrating conditions would not be expected at a first glance—provided 5a-nitro-α-tocopheryl acetate (**11**) or its model **12**, respectively.[11] This reaction and its mechanism were studied comprehensively and were shown to proceed via trioxaphenanthrene derivatives involving the acetyl group which is retained during the reaction.[12] Both the reaction of α-tocopherol and the reaction of α-tocopheryl acetate require rather drastic conditions to proceed, such as the use of concentrated nitric acid, and are not relevant for conversions under physiological conditions. The reactions are listed for the α-homologue **1**, its acetate **1a**, as well as for the truncated model compounds **5** and **5a** in Scheme 1, for the reason of comprehensiveness.

We were interested in the interaction of tocopherols with peroxidases and myeloperoxidases in different systems producing potent electrophiles or electrophile precursors, such as peroxynitrite, hypohalite, or thiocyanate. The tocopherols were thought to act as molecular probes to establish whether the

SCHEME 1. Nitration Reaction of α-Tocopherol (1) and α-Tocopheryl Acetate (1a) as well as Their Short-Chain Analogues 5 and 5a



enzymes exerted a purely nitrating (halogenating) effect or whether the electrophile production was always coupled to an oxidative effect. From the type and ratio of substitution/oxidation products, the conclusion about the mechanism was expected to be rather straightforward. In addition, these in vitro actions of the tocopherols under physiological conditions would also correspond to their in vivo reaction mode with electrophiles.

The interaction of non-α-tocopherols with nitrous and nitric acid, NO, and peroxynitrite was selected as the first system to study, prior to hypohalites or rhodanide. At this point, we were facing some problems: no data about nitration products of β- and δ-tocopherol existed, and more than five different sets of analytical data—with sometimes severely differing analytical (UV, NMR) data—existed in the literature for the nitration product of γ-tocopherol.[13−19] This left us with the task to establish (for β- and δ-tocopherol) or reinvestigate (for γ-tocopherol) the structure of the respective nitration products and to provide a reference set of analytical data to end the "uncertainty" found in the literature. For maximum clarity, the analytical data should be based on crystal structure analysis. Provided this technique established the structure of a compound, the corresponding set of analytical data, such as the NMR, MS, and UV spectra, would not be ambiguous and could be used as reference. As tocopherol derivatives with their isoprenoid side chain cannot be crystallized—or only in quality insufficient for X-ray analysis—we thus used truncated model compounds (**5**−**8**) possessing a methyl group instead of the isoprenoid side chain. The replacement of the side chain has no effect on the UV data and the aromatic NMR resonances.[20,21]

In this paper, we thus state the structure of the nitration products of the non-α-tocopherols, and we report their comprehensive analytical data (crystal structure, NMR, MS, UV)

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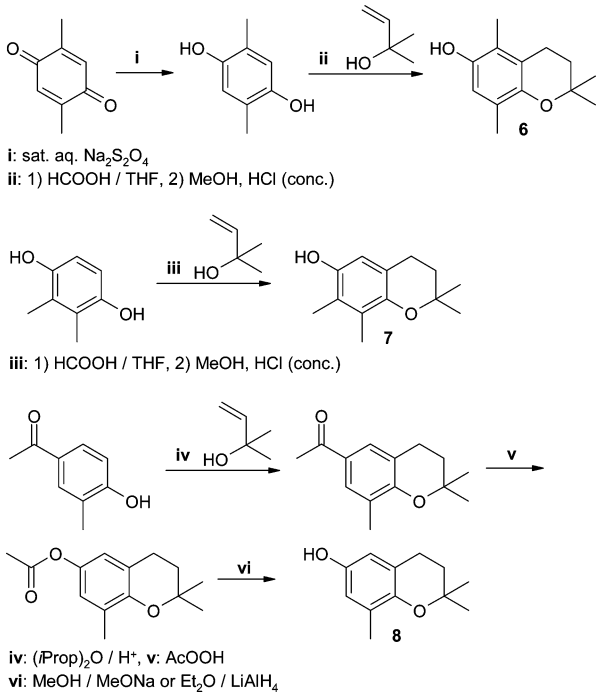
SCHEME 2. Synthesis of the Model Compounds 6−8



as reference for further work. The issue of nitration/nitrosation reactions of γ-tocopherol is reinvestigated, and the reason for the different results, as found so far for this reaction in the literature, is discussed. In addition, mono- versus bisnitration for δ-tocopherol is studied.

2. Results and Discussion

2.1. Synthesis of Truncated Tocopherol Model Compounds. The synthesis of the tocopherol model compounds having a methyl group instead of the isoprenoid side chain has been reported in the literature, and the α-tocopherol model (**5**) is available commercially. We started from these reports and optimized the procedures as detailed in the Experimental Section. A summary of the synthetic pathways is given in Scheme 2, and the crystal structures of the products are shown below within the schemes of the nitration reactions.

2.2. Nitration of β-Tocopherol. β-Tocopherol (**2**) and its model compound **6** are characterized by a nonsubstituted aromatic 7-C, which is also the position where nitration occurs. The aromatic proton in **2** and **6** resonated at 6.47 ppm in ^{1}H NMR ($CDCl_3$).[22] Compound **6** crystallized in the orthorhombic system with space group *Pbca*. In the solid state, the phenolic hydroxyl forms an intermolecular O−H−O hydrogen bond to the chroman oxygen of a neighboring molecule (O−O = 2.987(1) Å).

β-Tocopherol (**2**) is nitrated to form 7-nitro-β-tocopherol (**13**), a red colored, viscous oil. The nitration product of **6**, 2,2,5,8-tetramethyl-7-nitrochroman-6-ol (**14**), crystallized from methanol as red prisms in the monoclinic crystal system form with space group $P2_1/n$. In the solid state, the compound possesses a strong intramolecular hydrogen bond between the phenolic hydroxyl and one NO_2 oxygen in the *ortho*-position. The hydrogen bond distance O(2)−O(4) is 2.523(2) Å, and the bond is distinctly bent, O(2)−H(2O)−O(4) = 143.5°. This strong hydrogen bond

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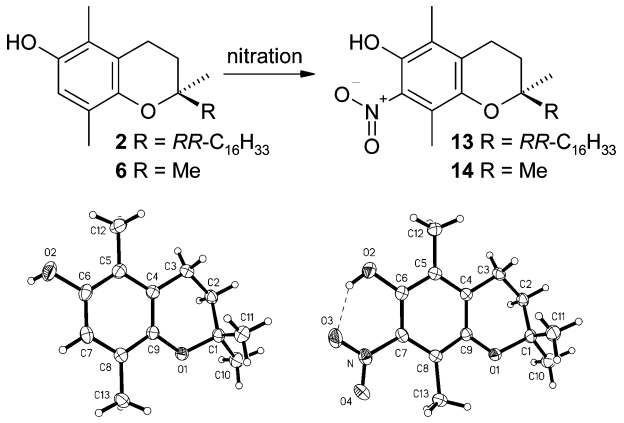
SCHEME 3. Nitration of β-Tocopherol (2) to 7-Nitro-β-tocopherol (13) and Analogous Reaction of Model Compound 6: Crystal Structure of 6 and Its Nitration Product 14



is retained in solution, with the proton resonance ($CDCl_3$) at 9.75 ppm being highly indicative of such a structural feature. The nitration reaction along with the crystal structures of both the starting material and the corresponding nitration product are shown in Scheme 3.

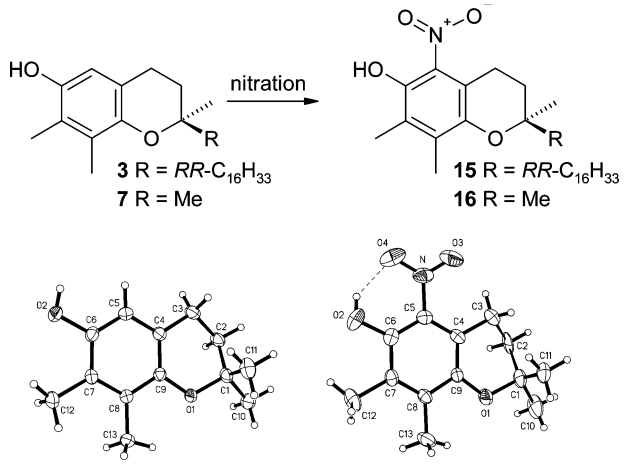
One peculiarity in the ^{13}C NMR spectra of the nitrotocopherols and nitrochromanols should be mentioned here; it is found also for compounds **15**, **16**, **19**, **20**, **22**, and **23**; see below. In common NMR solvents, such as chloroform and DMSO, only four distinct resonances for the aromatic system were found; the resonances of the two carbons bearing the phenolic hydroxyl and the nitro group in the *ortho*-position were either covered by background noise or appeared as rather broad signals (5−10 ppm). This behavior, which is well-known for *ortho*-nitrophenols and *para*-nitrophenol derivatives,[23] is due to nitrophenol−*aci*-nitrophenolate tautomerism. One way to overcome the "missing signals" is to record the spectra in strongly acidic solvent mixtures, such as that obtained by addition of 1% DCl to the NMR solvent or, alternatively, by measurement in DCOOD. If the compounds are sensitive toward acids, the nitrophenol can be converted into the corresponding phenolate which, however, changes the resonances quite drastically.

2.3. Nitration of γ-Tocopherol. By analogy to the β-isomer, γ-tocopherol (**3**) and its model compound **7** possess one free aromatic ring position, but this time located at C-5. The NMR resonance of the aromatic proton was found at 6.37 ppm.[22] The γ-model **7** crystallized in the monoclinic system, space group *C*2/*c*, and contained two independent molecules per asymmetric unit. The four molecules are linked by O−H−O hydrogen bonds donated and accepted by the phenolic OH groups to form a hydrogen bond ring with O−O distances of 2.726(2)−2.738(2) Å.

The interaction of γ-tocopherol with nitrosating and nitrating electrophiles has been studied,[13−19] and the formation of 5-nitro-γ-tocopherol (**15**) as the product of the nitration has been described in the literature. It was not demanding to repeat this finding. By analogy, the reaction of γ-model **7** under nitrating conditions proceeded neatly and readily to afford 2,2,7,8-tetramethyl-5-nitrochroman-6-ol (**16**). For the nitration reaction as well as the crystal structures of starting material and product,

(23) Pouchert, C. J.; Behnke, J. *The Aldrich Library of ^{13}C and ^{1}H FT NMR Spectra*, 1st ed.; Aldrich Chemical Company, 1993, pp 675A−676D for nitrosophenols and 678A−678C, 680C, and 681C for nitrophenols.

SCHEME 4. Nitration of γ-Tocopherol (3) to 5-Nitro-γ-tocopherol (15) and Analogous Reaction of Model Compound 7: Crystal Structure of 7 and Its Nitration Product 16



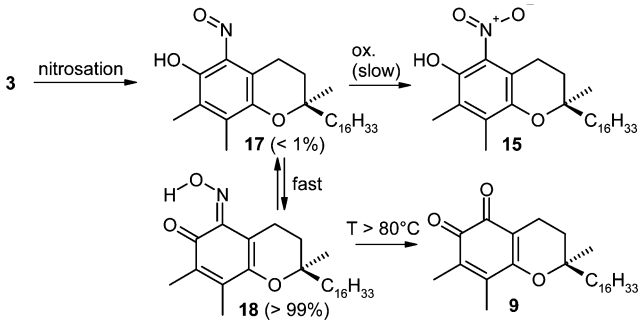
see Scheme 4. The compound was found to crystallize at room temperature in the orthorhombic system with a distinctly disordered structure, with the pyran ring adopting two orientations mirror-related relative to the mean plane of the benzene ring. Similar to the nitration product of β-tocopherol, also the *ortho*-nitro derivative of γ-tocopherol exhibited a strong intramolecular hydrogen bond from the phenolic hydroxyl to the nitro group, which remains intact in solution (proton resonance at 10.60 ppm).

It should be noted that 5-nitro-γ-tocopherol (**15**) is also a byproduct in nitrosation reactions of γ-tocopherol.[7−13] This chemistry has recently been studied in more detail, and preparation as well as analytical data of 5-nitroso-γ-tocopherol (**17**) have been described.[24] It was shown that 5-nitroso-γ-tocopherol (**17**), the initial product of the nitrosation, is immediately converted into its more stable tautomer upon contact with aqueous media, the 5-*O*-oxime of 5,6-*ortho*-tocopherylquinone (**18**), the equilibrium concentration of **17** being below 1%. However, the nitroso form was obtained in neat form by aprotic nitrosation of an organomercury derivative. Nitroso compound **17** is slowly oxidized in the presence of air under ambient conditions, affording 5-nitro-γ-tocopherol (**15**); see Scheme 5. Thus, also after consumption of the initially present nitrosating species, nitrosation mixtures of γ-tocopherol (**3**) or its model compound **7** are not stable and will finally afford the nitration product **15** or **16**, respectively. An additional process, proceeding at elevated temperatures, was identified: the release of hydroxylamine from quinone oxime **18** to afford the free *ortho*-quinone **9** (Scheme 5).[21]

The clear distinction of the nitroso and quinone monoxime compounds from the nitro derivatives **14** and **15**, along with the full analytical data set of the latter, will help to overcome the inconsistency and untrustworthiness of the analytical data for this compound found in the literature and help to correctly identify the compound when encountered in biological samples.

2.4. Nitration of δ-Tocopherol. δ-Tocopherol (**4**) is the only tocopherol with more than one free aromatic position, and both positions *ortho* to the phenolic hydroxyl, C-5 and C-7, are

SCHEME 5. Nitrosation of γ-Tocopherol and Further Reactions of the Primary Product 17 by Slow Oxidation to Nitro Compound 15 or by Fast Tautomerism to Benzoquinone Monoxime Intermediate 18 with Subsequent Formation of *ortho*-Quinone 9



nonsubstituted.[25] The aromatic protons resonated at 6.30 and 6.38 ppm with a coupling constant of 2.9 Hz. Several attempts to study the solid-state structure of **8** by X-ray single-crystal diffraction were unsuccessful because of absolutely unsuitable crystal qualities. Therefore the *p*-nitrobenzoate derivative (**8a**) was used instead (cf. Scheme 6). With regard to nitration studies, two questions were of interest. First, does bisnitration always occur, and second, if it was possible to obtain a mononitration product, is there a preference for one or the other *ortho*-position?

The product of the nitration of δ-tocopherol with two or more equivalents of nitrating agent was the dinitro derivative **19**, in the case of model compound **8** the corresponding dinitrochromanol **20**. Obtaining the mononitration product exclusively was not possible; the bisnitration product was always formed as byproduct, even if the phenol was applied in large excess relative to the nitrating agent. Varying temperature, solvents, and reagent concentrations had no large effect of the ratio between mono- and bisnitration. Always mixtures of mononitro and dinitro derivatives were obtained along with non-nitrated starting material. Evidently, the deactivating effect of the first nitro group on the aromatic system was surprisingly small so that the reactivity of the mononitration product toward nitronium species was not drastically decreased as compared to the non-nitrated starting material. The effect of the tocopherol substitution pattern, increasing electron density by inductive and mesomeric effects, was evidently able to largely compensate the −M effect of the first nitro substituent. Mononitration of δ-tocopherol occurred with complete selectivity at C-5, but not at C-7, affording 5-nitro-δ-tocopherol (**21**) from δ-tocopherol (**4**) and compound **22** from the truncated analogue **8** (Scheme 6).

The mononitration product **22** crystallized from methanol as orange, triclinic prisms, space group $P\bar{1}$. In the solid state, also this compound possesses a strong intramolecular hydrogen bond between the phenolic hydroxyl and one oxygen of the *ortho*-nitro group. In solution, this bond is reflected by the NMR proton resonance at 9.80 ppm. The hydrogen bond distance O(2)−O(4) is 2.527(2) Å, and the bond is distinctly bent, O(2)−H(20)−O(4) = 142.4°, closely similar to compound **14**. In solution, the presence of the hydrogen bridge is indicated by the 9.80 ppm proton resonance. Similar to **14**, the nitro group is not perfectly coplanar with the phenyl ring rather than inclined by an out-of-plane rotation of ca. 13° around the C(15)−N(1) bond in order to diminish steric congestion. In dinitrate **20**, the

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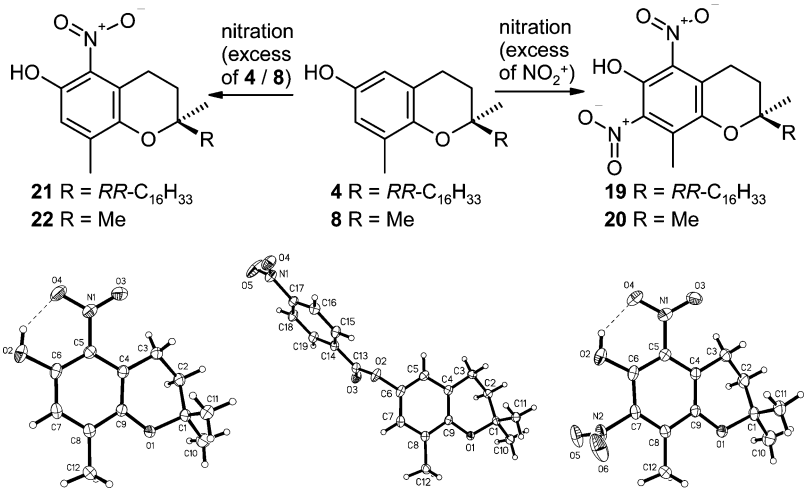
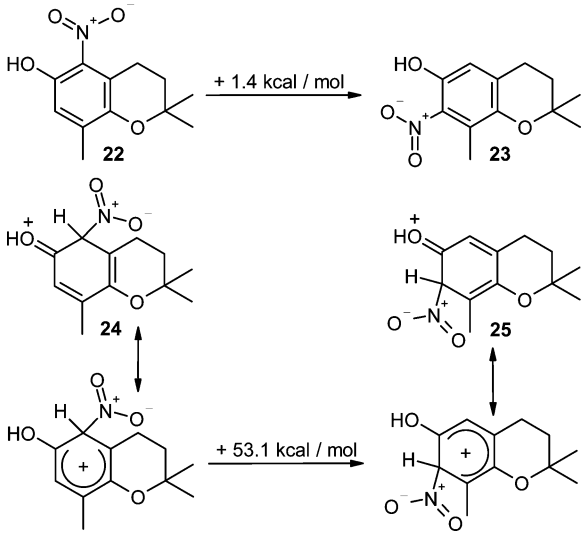
SCHEME 6. Nitration of δ-Tocopherol (4) to 5,7-Dinitro-δ-tocopherol (19) and 5-Nitro-δ-tocopherol (21) and Analogous Reaction of Model Compound 8: Crystal Structure of 8 (as the *p*-nitrobenzoate 8a) and Its Nitration Products 20 and 22



OH group is hydrogen bonded to the same nitro group as in mononitrate **22**, but here the inclination angle of the nitro group $N(1)O_2$ is approximately 24°, and the hydrogen bond distance O(2)−O(4) = 2.575(1) Å is larger, consequently. The second nitro group, $N(2)O_2$, is approximately perpendicular to the phenyl ring with an inclination angle to the phenyl ring of approximately 81° because of steric interference with the adjacent OH group and the adjacent $C(12)H_3$ group.

The selectivity in the nitration in favor of the 5-derivative was quite surprising at the first glance. In **4** and **8**, there are two free *ortho*-positions available which have no evident differences with regard to electronic effects or spatial conditions. With regard to steric hindrance, one could even assume the 7-position with a rotating methyl group (C-7a) to be slightly better accessible than the 5-position having an inflexible heterocyclic methylene neighbor (C-4). The selectivity also cannot be explained by the stability of the products: the ZPE-corrected total energies of the 5-nitro derivative **22** and the hypothetical 7-nitro derivative **23**, computed at the MP-2/6-31G(d,p)//B3LYP/6-31G(d,p) level of theory, differ only by 1.4 kcal/mol, which is almost within the error limit. Instead, the observed imbalance in the product distribution can be accounted for by the stability of the primary nitration intermediates, the nitrocyclohexadienyl cations or σ-complexes. The 5-nitro intermediate **24** was calculated to be by 53.1 kcal/mol more stable than the corresponding 7-nitro counterpart (**25**), which agreed perfectly with the observed dominance of the 5-nitro-product (Scheme 7). The energy difference can be readily explained by the theory of strain-induced bond localization (SIBL), which had been successfully applied to clarify the frequently observed preference of the α-tocopherol-derived 5-*ortho*-quinone methide (the "up"-oQM) over the isomeric 7-*ortho*-quinone methide (the "down"-oQM).[26,27] The same argument as used for those *ortho*-quinone methides can be applied for the nitration intermediates, which in fact also represent quinoid systems. A brief explanation shall be given in the following—a detailed explanation of the theory is not in line with the scope of this report: the sum of the annulation angles governs the stability and thus the ratio of the two isomeric

SCHEME 7. Calculated ZPE-Corrected Energy Differences between 5-Nitro-δ-tocopherol Model 22 and its Hypothetical 7-Nitro Isomer 23, and between the Two σ-Complexes (24 and 25) Leading to Those Nitro Derivatives



quinoid structures. For tocopherol and other 2,2-dialkyl-substituted chroman-6-ol derivatives, the annulation angle sum is approximately 242°, and the ratio between the two *ortho*-quinone methides was experimentally determined to be about 97/3 in favor of the 5-*ortho*-quinone methide, in complete agreement with computations. The computationally predicted ratio for the two isomeric nitration intermediates **24** and **25** was 98.2/1.8 in favor of the 5-nitro isomer. Experimentally, no 7-nitro compound was retrieved and the 5-nitro derivative was formed exclusively.

With regard to nitrosation, the chemistry of δ-tocopherol largely equaled that of the γ-homologue, although there were some quantitative differences (Scheme 8). To obtain the 5-nitroso-δ-tocopherol (**26**) nitroso derivative in neat form, a similar detour involving an organomercurial derivative that was nitrosated under aprotic conditions was necessary; a 7-nitroso derivative was not obtained. Similar to 5-nitroso-γ-tocopherol (**17**), also 5-nitroso-δ-tocopherol (**26**) tended to form the tautomeric benzoquinone monoxime **27** immediately under protic conditions. The equilibrium was not as far on the side of

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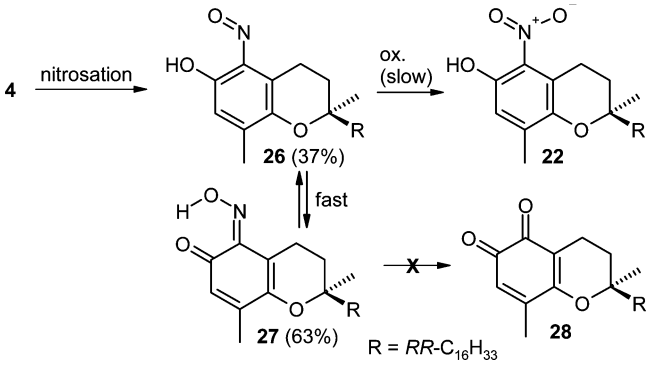
SCHEME 8. Nitrosation of δ-Tocopherol and Further Reactions of the Primary Product 26 by Slow Oxidation to Nitro Compound 22 or by Fast Tautomerism to Benzoquinone Monoxime Intermediate 27 without Subsequent Formation of *ortho*-Quinone 28



the quinone in the case of the γ-homologue, the nitroso-to-quinone oxime ratio being 37/63, in contrast to a >99/<1 ratio for the γ-system. Also, **26** was slowly oxidized in air to 5-nitro-δ-tocopherol (**22**), similar to 5-nitroso-γ-tocopherol (**17**) giving 5-nitro-γ-tocopherol (**15**). However, the quinone oxime tautomer **27** proved to be completely stable upon heating, showing no signs of a loss of hydroxylamine and thus no conversion into the corresponding *ortho*-quinone.

3. Conclusions

The nitration of all three non-α-tocopherols has been studied. The respective products were comprehensively analytically characterized. Crystal structures of the truncated chromanol model compounds and of all nitration products were obtained and used to unambiguously confirm the structures. All nitration products are characterized by strong hydrogen bridges between phenolic hydroxyl and the neighboring *ortho*-nitro substituent, both in crystalline phase and in solution. The nitration products of β- and δ-tocopherol were reported for the first time. The nitration/nitrosation system of γ-tocopherol and δ-tocopherol was revised showing the systems to involve an equilibrium between nitroso compound and tautomeric *ortho*-quinone monoxime, the former being slowly oxidized into the corresponding nitro derivative in the presence of air.

A favoring of position 5 over position 7 was found for nitro compound formation in the case of δ-tocopherol, which has two free aromatic ring positions available for nitration. This behavior finds parallels in the oxidation chemistry of α-tocopherol, which showed a comparable preference for the formation of the *ortho*-quinone methide in the 5-position over that in the 7-position. This similarity is reasonable since, in both cases, the product distribution is determined by the stabilities of quinoid intermediates (σ-complexes), which can be readily explained according to the theory of strain-induced bond localization (SIBL).

With the present study, we would like to provide a reference set of analytical data which we trust will be helpful in all kinds of related enzymatic, in vitro and in vivo studies involving reactions of tocopherols and tocopherol model compounds with nitrogen-derived electrophiles, such as nitrating species, NO_x, nitroso compounds, or peroxynitrite. We also hope that the clarification of the nitration/nitrosation chemistry of the tocopherols, as an important class of bioactive compounds, will be useful with regard to nutritional, biological, and medicinal studies where there is a frequent need for reference data from neat standard compounds for comparison.

4. Experimental

4.1. Synthesis and Compound Characterization of Model Compounds. 4.1.1. Synthesis of β-Tocopherol Model Compound 6. 2,5-Dimethyl-1,4-hydroquinone. A solution of 2,5-dimethyl-1,4-benzoquinone (0.50 g, 3.67 mmol) in diethyl ether (20 mL) was shaken with a freshly prepared saturated aqueous solution of $Na_2S_2O_4$ (40 mL). The colorless ether phase was washed with water, dried over $MgSO_4$, and concentrated in vacuo. The residue was triturated with light petroleum ether (30 mL). After cooling, a white precipitate of 2,5-dimethyl-1,4-hydroquinone (0.42 g, 83%) was obtained:[28,29] mp = 122−124 °C; TLC R_f = 0.50 (*n*-hexane/diethyl ether, v/v = 5:5); ^{1}H NMR (CD_3OD) δ 2.10 (s, 6H, CH_3), 6.50 (s, 2H, ^{Ar}CH); ^{13}C NMR (MeOD) δ 15.90 (CH_3), 118.12 (^{Ar}CH), 123.33 (^{Ar}C), 148.94 (^{Ar}C−OH). Anal. Calcd for $C_8H_{10}O_2$: C, 69.55; H, 7.30; O, 23.16. Found: C, 69.71; H, 7.34.

6-Hydroxy-2,2,5,8-tetramethylchroman (6). Into a solution of 2,5-dimethyl-1,4-hydroquinone (see above, 1.50 g, 10.85 mmol) in formic acid (14.03 mL, 98−100%) and THF (3.74 mL) was added dropwise 2-methyl-3-buten-2-ol (10.81 mmol, 1.13 mL) in THF (0.93 mL) under stirring at 100 °C. The addition was completed within 1 h. The mixture was further stirred for 3 h, cooled to rt, and poured into crushed ice. Water was added, and the mixture was extracted with diethyl ether. Light petroleum ether (10 mL) was added to the combined ethereal extracts, and the mixture was washed with water. This way, most of the formic acid contained in the ether phase was removed. The solvent was evaporated, and the residue was dissolved in methanol (14 mL). Concentrated HCl (0.18 mL) was added, and the solution was heated at 60 °C for 20 min to hydrolyze any formate ester that might have formed. The methanol was evaporated, and the residue was dissolved in diethyl ether (10 mL). After washing with water, with a saturated aqueous solution of $NaHCO_3$ and again with water and drying over $MgSO_4$, the mixture was concentrated in vacuo. The residue was triturated with light petroleum ether under reflux (40 mL) for 15 min. After cooling to rt, nonreacted 2,5-dimethyl-1,4-hydroquinone (∼15%) was filtered off, and the filtrate was concentrated in vacuo, purified by flash chromatography (gradient *n*-hexane/diethyl ether, v/v = 9:1 → *n*-hexane only), and recrystallized from *n*-hexane to afford a white crystalline solid (**6**) in 30% overall yield:[30−33] mp = 79−80 °C; TLC R_f = 0.35 (light petroleum ether/diethyl ether, v/v = 8:2); ^{1}H NMR δ 1.28 (s, 6H, H-2a), 1.79 (t, 2H, ^3J = 6.8 Hz, H-3), 2.08 (s, 3H, H-5a/8b), 2.10 (3H, s, H-8b/5a), 2.61 (t, 2H, ^3J = 6.8 Hz, H-4), 4.16 (s, 1H, OH), 6.47 (s, 1H, H-7); ^{13}C NMR δ 11.35 (C-5a), 16.21 (C-8b), 21.52 (C-4), 27.07 (C-2a, d.i.), 33.29 (C-3), 72.86 (C-2), 115.70 (C-7), 119.57 (C-5), 120.52 (C-4a), 124.45 (C-8), 146.10 (C-6), 146.52 (C-8a).

Also colorless crystals of the bisalkylated byproduct 2,2,5,7,7,-10-hexamethyl-2,3,4,7,8,9-hexahydro-pyrano[2,3-*g*]chromene were isolated in 9% yield: mp 168−170 °C; TLC R_f = 0.64 (SiO_2; *n*-hexane/diethyl ether, v/v = 9:1); ^{1}H NMR δ 1.28 (s, 12H, CH_3), 1.77 (t, 4H, ^3J = 6.8 Hz), 2.04 (s, 6H, ^{Ar}C−CH_3), 2.60 (t, 4H, ^3J = 6.8 Hz).

Compound **6** was recrystallized from *n*-hexane to obtain crystals suitable for X-ray crystallography.

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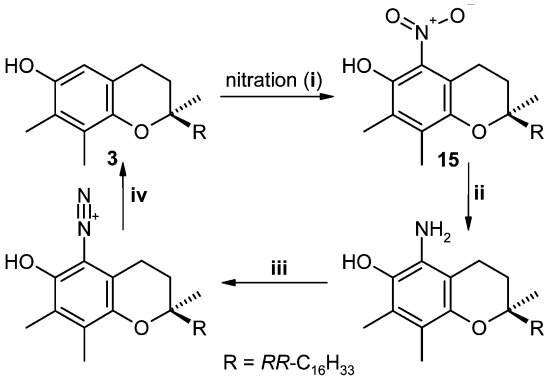
SCHEME 9. Confirmation of the Retention of (2*R*,4′*R*,8′*R*)-Stereochemistry upon Nitration of γ-Tocopherol (3)



4.1.2. Synthesis of γ-Tocopherol Model Compound 7. 6-Hydroxy-2,2,7,8-tetramethylchroman (7). A solution of 2-methyl-3-buten-2-ol (10.81 mmol, 1.13 mL) in THF (0.93 mL) was added dropwise to a solution of 2,3-dimethyl-1,4-hydroquinone (1.50 g, 10.85 mmol) in formic acid (14.0 mL, 98−100%) and THF (3.7 mL), under stirring at 100 °C. The addition was completed within 1 h. Stirring was continued for additional 3 h, and the cooled mixture was poured onto crushed ice.

Water was added, and the mixture was extracted with diethyl ether. Light petroleum ether (10 mL) was added to the combined ethereal extracts, and the mixture was washed with water. This way, most of the formic acid contained in the ether phase was removed. The solvent was evaporated, and the residue was dissolved in methanol (14 mL). Concentrated HCl (0.18 mL) was added, and the solution was heated at 60 °C for 20 min to hydrolyze any formate ester that might have formed. The methanol was evaporated, and the residue was dissolved in diethyl ether (10 mL). After washing with water, with saturated aqueous solution of $NaHCO_3$, and again with water and drying over $MgSO_4$, the mixture was concentrated in vacuo. The residue was triturated with light petroleum under reflux (40 mL) for 15 min. After cooling to rt, nonreacted 2,3-dimethyl-1,4-hydroquinone (∼15%) was filtered off and the filtrate was concentrated in vacuo, followed by flash chromatography (gradient light petroleum ether/diethyl ether v/v = 9:2 → v/v = 9:0.8) to provide a light brown solid (**7**) in 30% yield:[15,30,33] mp = 75−77 °C; TLC R_f = 0.35 (light petroleum ether/diethyl ether, v/v = 8:2); ^{1}H NMR δ 1.29 (s, 6H, H-2a), 1.74 (t, 2H, ^3J = 6.8 Hz, H-3), 2.10 (s, 3H, H-7a/8b), 2.13 (3H, S, H-8b/7a), 2.68 (t, 2H, ^3J = 6.8 Hz, H-4), 4.0 (br, 1H, OH), 6.37 (s, 1H, H-5); ^{13}C NMR δ 11.85, 11.89 (C-7a, C-8b), 22.63 (C-4), 26.94 (C-2a), 32.93 (C-3), 73.39 (C-2), 112.13 (C-5), 118.05 (C-4a), 121.61 (C-7), 125.76 (C-8), 145.85 (C-8a), 146.25 (C-6); IR (KBr) 3271, 2979, 2927, 2359, 1619, 1420, 1253, 1208 cm^{-1}.

Also, slightly yellowish crystals of the bisalkylated product 3,3,5,6,8,8-hexamethyl-1,2,3,8,9,10-hexahydropyrano[3,2-*f*]-chromene were found (10%). Anal. Calcd for $C_{18}H_{26}O_2$: C, 78.79; H, 9.55; O, 11.66. Found: C, 78.88; H, 9.56.

Compound **7** was recrystallized from *n*-hexane with some drops of ethyl acetate to obtain crystals suitable for X-ray crystallography.

4.1.3. Synthesis of δ-Tocopherol Model Compound 8. 6-Acetyl-2,2,8-trimethylchroman. 2-Methyl-3-buten-2-ol (26.6 mmol, 2.29 g, 4 equiv) was added dropwise to a solution of 4-hydroxy-3-methylacetophenon (1.00 g, 6.65 mmol) in *iso*-propylether (10 mL) and concd H_2SO_4 (0.33 mL), under stirring at reflux (55−58 °C). The addition was completed within 3 h, and the mixture was further stirred for additional 3 h. After cooling to rt, the reaction mixture was washed with water, saturated aqueous $NaHCO_3$ solution, and again with water, dried over $MgSO_4$, and concentrated in vacuo. The yellow, oily residue was crystallized from light petroleum ether to give 6-acetyl-2,2,8-trimethylchroman as an off-white solid (1.02 g, 70%): mp = 60−62 °C; TLC R_f = 0.30 (*n*-hexane/diethyl ether, v/v = 7:3), orange color with *p*-anisaldehyde reagent; ^{1}H NMR δ 1.28 (s, 6H, H-2a), 1.75 (t, 2H, ^3J = 6.76 Hz, H-3), 2.12 (s, 3H, H-8b), 2.45 (s, 3H, −$COCH_3$), 2.74 (t, 2H, ^3J = 6.76 Hz, H-4), 7.51 (s, 1H, Ar−H), 7.53 (s, 1H, Ar−H); ^{13}C NMR δ 16.08 (C-8b), 22.52 (C-4), 26.21 (*C*H_3CO), 27.08 (C-2a, d.i.), 32.53 (C-3), 75.31 (C-2), 119.86, 126.30, 128.26, 128.56, 128.91 (C-4a, C-5, C-6, C-7, C-8), 156.84 (C-8a), 197.30 (CO).

6-Acetoxy-2,2,8-trimethylchroman. Into a mixture of 6-acetyl-2,2,8-trimethylchroman (0.47 g, 2.16 mmol), glacial acetic acid (3.75 mL), and water (0.70 mL) was added peracetic acid (∼39% in acetic acid, 0.70 mL) at rt. The mixture was left standing overnight and then poured into water. Residual peracid was decomposed with sodium hydrogensulfite. The reaction mixture was extracted with ethyl acetate and subsequently washed with water, saturated aqueous $NaHCO_3$ solution, and again water, dried over $MgSO_4$, and concentrated in vacuo. The yellow, oily residue obtained was purified by flash chromatography (*n*-hexane with a few drops of diethyl ether) to afford 6-acetoxy-2,2,8-trimethylchroman as a colorless viscous liquid (0.31 g, 60%): TLC R_f = 0.34 (*n*-hexane/diethyl ether, v/v = 8:2); ^{1}H NMR δ 1.39 (s, 6H, H-2a), 1.84 (t, 2H, ^3J = 6.77 Hz, H-3), 2.21 (s, 3H, H-8b), 2.32 (s, 3H, acetyloxy), 2.81 (t, 2H, ^3J = 6.74 Hz, H-4), 6.70 (s, 1H, Ar−H), 6.74 (s, 1H, Ar−H); ^{13}C NMR δ 16.10 (C-8b), 21.07 (CH_3, acetyloxy), 22.76 (C-4), 27.05 (C-2a, d.i.), 32.57 (C-3), 74.04 (C-2), 119.04, 120.70, 121.12, 127.28 (C-4a, C-5, C-7, C-8), 142.50 (C-6), 149.85 (C-8a), 170.20 (COO, acetyloxy).

6-Hydroxy-2,2,8-trimethylchroman (8). Procedure A: Into the ice-cooled solution of acetoxy-2,2,8-trimethylchroman (2.66 g, 11.38 mmol) in dry methanol (120 mL) was added dropwise a 0.1 M sodium methanolate in methanol (0.1 M, 2.0 equiv, 228 mL, 22.8 mmol), and the mixture was stirred for 15 min at rt. After completion of the reaction (TLC control), the mixture was diluted with methanol, and strongly acidic cation exchange resin (Dowex 50WX8) was added until neutralization. The mixture was filtered, and the filtrate concentrated in vacuo. The residue was purified by flash chromatography (gradient *n*-hexane/diethyl ether v/v = 6:1.5 → v/v = 6:1) and recrystallization from *n*-hexane to afford 6-hydroxy-2,2,8-trimethylchroman (**8**) as a colorless solid in 97% yield. **Procedure B:** A suspension of $LiAlH_4$ (47.7 mg, 1.25 mmol) in dry diethyl ether (2.8 mL) was refluxed (bath temperature 42 °C) for an hour. 6-Acetoxy-2,2,8-trimethylchroman (0.27 g, 1.18 mmol) dissolved in dry diethyl ether (0.7 mL) was added dropwise under stirring at reflux. The addition was completed within 1 h, the stirring was continued for 2 h, and the mixture was cooled to rt. Excess hydride was destroyed by the dropwise addition of wet diethyl ether. The mixture was refluxed for another 10 min and cooled to rt. At this stage, there should be no gray, nonreacted $LiAlH_4$ remaining and the precipitate should be bright white. The reaction mixture was neutralized with 5% aqueous H_2SO_4 solution, and phases were separated. The organic layer was washed with water, with saturated aqueous $NaHCO_3$ solution, again with water, dried over $MgSO_4$, and evaporated in vacuo. The remaining pale brown, oily residue was purified as described in procedure A (88%):[34] mp 81−82 °C; TLC R_f = 0.34 (*n*-hexane/diethyl ether, v/v = 6:4); ^{1}H NMR δ 1.21 (s, 6H, H-2a), 1.66 (t, 2H, ^3J = 6.8 Hz, H-3), 2.03 (s, 3H, H-8b), 2.60 (t, 2H, ^3J = 6.8 Hz, H-4), 4.66 (br, 1H, OH), 6.30 (d, 1H, ^4J = 2.9 Hz, H-7), 6.38 (d, 1H, ^4J = 2.9 Hz, H-5); ^{13}C NMR δ 16.02 (C-8b), 22.80 (C-4), 26.89 (C-2a), 32.84 (C-3), 73.54 (C-2), 112.64 (C-5), 115.68 (C-7), 121.07 (C-

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TABLE 1. Crystal Data, Data Collection, and Refinement Details for 6, 7, 8a, 14, 16, 20 and 22

	6	7	8a	14	16	22	20
formula	$C_{13}H_{18}O_2$	$C_{13}H_{18}O_2$	$C_{19}H_{19}NO_5$	$C_{13}H_{17}NO_4$	$C_{13}H_{17}NO_4$	$C_{12}H_{15}NO_4$	$C_{12}H_{14}N_2O_6$
mol wt	206.27	206.27	341.35	251.28	251.28	237.25	282.25
color, habit	colorless, oval	colorless, block	yellow, plate	red, prism	orange, plate	orange, prism	orange, plate
symmetry, space group	orthorhombic *Pbca*	monoclinic, *C*2/*c*	monoclinic, $P2_1/c$	monoclinic, $P2_1/n$	orthorhombic, $Pna2_1$	triclinic, $P\bar{1}$	monoclinic, $P2_1/n$
a, Å	15.5426(8)	22.4807(12)	16.2752(14)	8.8349(13)	11.139(5)	6.7561(14)	10.4534(8)
b, Å	8.5151(4)	10.3086(5)	11.8402(10)	7.2041(11)	16.477(7)	9.3500(19)	14.3766(11)
c, Å	17.1816(8)	22.0953(12)	8.8053(8)	19.113(3)	7.087(3)	9.6965(19)	9.4672(7)
α, deg	90	90	90	90	90	108.101(4)	90
β, deg	90	114.706(1)	97.757(2)	94.448(3)	90	91.717(4)	114.346(2)
γ, deg	90	90	90	90	90	96.312(4)	90
V, Å^3	2273.9(2)	4651.8(4)	1681.3(3)	1212.8(3)	1300.8(9)	577.3(2)	1296.3(2)
Z	8	16	4	4	4	2	4
D_{calcd}, g cm^{-3}	1.205	1.178	1.349	1.376	1.283	1.365	1.446
μ, mm^{-1}	0.079	0.078	0.098	0.102	0.095	0.103	0.118
θ range, deg	2.6−27.0	2.3−27.0	2.5−30.0	2.5−27.0	3.9−27.0	2.3−27.0	2.6−27.0
temp, K	173(2)	173(2)	100(2)	173(2)	297(2)	173(2)	173(2)
data collected	13178	14100	16693	5521	2645	4048	7067
unique data	2464 [R_{int} = 0.021]	5033 [R_{int} = 0.019]	4739 [R_{int} = 0.026]	2601 [R_{int} = 0.055]	2160 [R_{int} = 0.039]	2419 [R_{int} = 0.016]	2799 [R_{int} = 0.019]
no. of params/restraints	140/41	285/82	229/67	168/49	191/76	157/46	185/54
$R1^a$ ($F^2 > 2\sigma(F^2)$)	0.0420	0.0462	0.0429	0.0430	0.0489	0.0495	0.0409
$wR2^b$ (all data)	0.1209	0.1309	0.1231	0.1146	0.1303	0.1370	0.1232

^{a} $R1(F) = \sum||F_o| - |F_c||/\sum|F_o|$. ^{b} $wR2(F^2) = \{\sum[w(F_o^2 - F_c^2)^2]/\sum[(w(F_o^2)^2]\}^{1/2}$

4a), 127.29 (C-8), 146.09 (C-8a), 147.66 (C-6); HRMS (ESI Q-TOF) *m*/*z* calcd for $C_{12}H_{16}O_2$ $[MH]^+$ 193.1223, found $[MH]^+$ 193.1284.

***p*-Nitrobenzoate of *δ*-Tocopherol Model Compound (8a).** *p*-Nitrobenzoyl chloride (0.24 g, 1.3 mmol) and DMAP (0.095 g, 0.78 mmol) were added to the solution of 6-hydroxy-2,2,8-trimethylchroman (**8**, 50 mg, 0.26 mmol) in dichloromethane (26 mL). The solution was stirred at rt under N_2 for 2 h, triethylamine (0.36 mL, 2.6 mmol) was added, and stirring was continued for 3 h. The solvent and excess triethylamine were removed under vacuum, and the remainder was redissolved in dichloromethane and washed with a 5% aq $NaHCO_3$, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by flash chromatography (*n*-hexane/diethyl ether, v/v = 9:0.5) to give 2,2,8-trimethylchroman-6-yl *p*-nitrobenzoate (**8a**) in 94% yield: TLC R_f = 0.42 (*n*-hexane/diethyl ether, v/v = 8:2). The product was recrystallized twice from methanol to obtain crystals suitable for X-ray crystallography.

4.2. Synthesis and Compound Characterization of Nitration Products. General Procedure for the Nitration of Non-α-tocopherols and Model Compounds. Into the stirred solution of tocopherol or tocopherol model compound (0.48 mmol) in absolute ethanol (45 mL) were added dropwise glacial acetic acid (1.8 mL) and aqueous $NaNO_2$ solution (2% w/v, 27 mL, for model compounds and 25% w/v, 2.0 mL, for tocopherols). After completion of the reaction, usually after 30 min at rt (TLC control), the orange reaction mixture was neutralized with aqueous KOH (20%, w/v), diluted with water (90 mL), and extracted three times with *n*-hexane. The combined organic extracts were washed with water and with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by flash chromatography with a short path of SiO_2 using *n*-hexane containing 2% of diethyl ether as the eluant.

This procedure has been described to provide good yields of nitrophenols,[13,15,16,18] giving yields superior to the direct nitration with nitric acid, which was confirmed in our study. Nitrite in acidic medium undergoes immediate disproportionation to nitric acid (2 equiv) and nitric oxide (1 equiv). The latter has an additional beneficial effect, acting as trap for oxygen by reaction to NO_2 and thus minimizing oxidative side reactions. The nitric acid generates the actual nitrating species NO_2^+ by protonation and cleavage of water.

In parallel, nitration was performed with HNO_3 as the nitrating agent according to the following procedure which generally gave the same nitration products as the above method, although the yields were generally lower by 5−12%. Into the stirred solution of tocopherol or tocopherol model compound (0.48 mmol) in absolute ethanol (45 mL) was added dropwise aqueous nitric acid (35%, 2 mL) in 5 mL of ethanol. After completion of the reaction, usually after 10 min at rt (TLC control), the orange reaction mixture was worked-up and the product purified as given above.

7-Nitro-*β*-tocopherol (13). *β*-Tocopherol (**2**) was used as the starting material providing 7-nitro-*β*-tocopherol (**13**) as an orange oil (64%): TLC R_f = 0.63 (*n*-hexane/diethyl ether, v/v = 9:1); ^{1}H NMR *δ* 1.83 (m, 2H, H-3), 2.18 (s, 3H, H-5a/8b), 2.36 (s, 3H, H-5a/8b), 2.70 (t, 2H, ^3J = 7.0 Hz, H-4), 10.32 (s, 1H, −OH); ^{13}C NMR *δ* 11.6 (C-5a), 13.1 (C-8b), 21.7 (C-4), 23.9 (C-2a), 31.4 (C-3), 75.2 (C-2), 120.4 (C-5), 123.3 (C-8), 129.9 (C-4a), 136.6 (C-8), 145.6, 145.6 (C-6, C-8a), isoprenoid side chain: 19.7 (C-4a′), 19.8 (C-8a′), 21.0 (C-2′), 22.7 (C-13′), 22.7 (C-12a′), 24.5 (C-6′), 24.7 (C-10′), 28.0 (C-12′), 32.6 (C-8′), 32.8 (C-4′), 37.3 (C-7′), 37.4 (C-9′), 37.5 (C-5′), 37.6 (C-3′), 39.3 (C-11′), 39.7 (C-1′). Anal. Calcd for $C_{28}H_{47}O_4N$: C, 72.84; H, 10.26; N, 3.03. Found: C, 72.93; H, 10.18; N, 2.99.

4.3. Stereochemical Integrity. For the nitration experiments described, stereochemically pure (2*R*,4′*R*,8′*R*)-tocopherols were used. To clarify a possible erosion of the stereochemistry under nitration conditions, *γ*-tocopherol (**3**) was nitrated according to three different procedures (Scheme 9): the above-given rather mild variant, a treatment with 1.2 equiv of concentrated HNO_3 (64−66%) in ethanol, and a treatment with NO_2BF_4 (5 equiv in diethyl ether followed by acidic workup), the latter two procedures applying deliberately drastic conditions. The obtained nitration product was reduced with Zn/AcOH/HCl to the amine, diazotated with $NaNO_2$, and the diazonium salt thermally reconverted into the starting tocopherol, the reactions following standard procedures (Scheme 9). The retrieved *γ*-tocopherol was prepurified by column chromatography (*n*-hexane/diethyl ether, v/v = 9:1) to afford 45−53% (relative to the starting *γ*-tocopherol) of possibly isomerized product, which was subsequently analyzed by chiral HPLC (Chiracel OD-H, 250 × 4.6 mm, *n*-hexane/EtOH, v/v = 99.5/0.5, 1.0 mL/min, UV detection at 220 nm). In no case—not even under those purposely harsh conditions—an epimerization at the chiral 2-C chroman center took place. It is therefore assumed that also in the reactions of *β*-tocopherol (**2**) and *δ*-tocopherol (**4**) the (2*R*,4′*R*,8′*R*)

stereochemistry was retained, although this has not been proven experimentally for those tocopherol homologues.

4.4. X-ray Structure Determination. X-ray data of compounds **6**, **7**, **8a**, **14**, **16**, **20**, and **22** were collected on a diffractometer using graphite-monochromated Mo Kα radiation ($\lambda = 0.71073$ Å) and 0.3° ω-scan frames covering hemispheres of the reciprocal space with $\theta_{max} = 27-30°$. After data integration, corrections for absorption, $\lambda/2$ effects, and crystal decay were applied.[35] The structures were solved by direct methods, completed by Fourier syntheses, and refined on F^2.[36] All non-hydrogen atoms were refined anisotropically. Most H atoms were placed in calculated positions and thereafter treated as riding. A torsional parameter was refined for each methyl group. Crystal data and experimental details are given in Table 1, and the molecular structures are shown in Schemes 2, 3, and 6. Bond lengths and angles are available in CIF format as Supporting Information.

It should be noted that the nitrated γ-tocopherol model compound **16** showed clear-cut disorder readily identifiable by weak Bragg scattering and strong diffuse scattering which did not disappear on reducing temperature. Depending on the structure refinement model, this disordered structure can alternatively be described by space group *Pnam* instead of $Pna2_1$, the latter of which was preferred on technical reasons in the present work.

The room temperature crystal structures of compounds **6** and **7** have been previously determined by other authors,[37] but the structure data deposited at The Cambridge Crystallographic Data Centre[38] need a correction in the case of **6** (CCDC refcode WELHAW; *a*-axis and *b*-axis were erroneously interchanged) and nonstandard unit cell settings for **6** and **7** (CCDC refcode WELHEA) were used in addition.

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Supporting Information Available: Complete crystallographic data and technical details in CIF format for compounds **6**, **7**, **8a**, **14**, **16**, **20** and **22**. Preparation and NMR data (^{1}H, ^{13}C) of 6-hydroxy-7-nitro-2,2,5,8-tetramethylchroman (**14**), 5-nitro-γ-tocopherol (**15**), 6-hydroxy-5-nitro-2,2,7,8-tetramethylchroman (**16**), 5,7-dinitro-δ-tocopherol (**19**), 6-hydroxy-5,7-dinitro-2,2,7,8-tetramethylchroman (**20**), 5-nitro-δ-tocopherol (**21**), and 6-hydroxy-5-nitro-2,2,8-trimethylchroman (**22**). Computational details for intermediates **24** and **25**. This material is available free of charge via the Internet at http://pubs.acs.org.

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