Synthesis and analytical characterization of monomeric N-oxidized derivatives of α -tocopheramine

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Abstract

α -Tocopheramine has shown great promises as a stabilizer for synthetic and natural polymers, but is also investigated in various biomedical scenarios. Many studies have been hampered by the fact that the oxidation products of α -tocopheramine have not yet been properly identified and their analytical data are still lacking. In the present study, we synthesized and fully analytically characterized all N-oxidation products that can form upon oxidation of α -tocopheramine in aqueous media, including the hydroxylamine, nitroso, and nitro derivative, in this way providing standards for the identification of the so far elusive byproducts. Synthesis and stability of the derivatives are discussed.

Graphic abstract

Keywords Tocopheramine · Vitamin E · Oxidation · Reduction · *ortho*-Quinone methide

Introduction

As early as 1942, α -tocopheramine (**1**), the amino analogue of α -tocopherol (**2**), the main component in vitamin E, has been reported, which differs from its vitamin E parent compound in the presence of an amino function instead of the phenolic OH group [1, 2]. Named “tocopheramines” or “aminotocopherols”, these aza-derivatives behave similarly to the tocopherols with regard to antioxidant activity, although their general oxidation chemistry can be different. Like tocopherols, tocopheramines are non-toxic and

biologically fully compatible [2, 3]. They have been studied both with regard to their biological activities, their antioxidant behavior and their general chemistry, but a wide use has been hampered by their limited availability, even if the related activities exceeded those of the corresponding tocopherols in some cases [4, 5]. While α -tocopherol is produced on a large industrial scale in well-optimized processes [6], α -tocopheramine still requires comparatively complex approaches which have not yet exceeded the pilot scale [7]. Tocopheramines have been evaluated as food [8] and feed additives [9], surfactants [10], and polymer stabilizers [11]. More recent research has shown amides derived from α -tocopheramine to possess promising anticancer and proapoptotic activities [12, 13] which were even superior to the well-studied, structurally related ester α -tocopheryl succinate [14–16], and effectiveness in ophthalmological and reproduction studies [17, 18]. Also, its activity in stabilizing cellulose solutions in *N*-methylmorpholine-*N*-oxide, as used on industrial scale to manufacture cellulosic manmade fibers in the lyocell process, was quite promising [19]. It was inert against the cellulose solvent and effectively counteracted

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both cellulose degradation and solvent decomposition [20]. It is, therefore, understandable that tocopheramines have become of greater interest in recent years from various points of view.

Oxidation of α -tocopheramine under aqueous conditions or in the presence of water affords α -tocopherylquinone (**3**) [21, 22] as the main product (Scheme 1), but it also generates byproducts, the structure of which is so far not known. Many studies with tocopheramines are hampered by this byproduct formation. Oxidation of α -tocopherol in aqueous medium affords the same main reaction product, compound **3**, but the side reactions and byproducts—mainly spiro-dimer **5** [23, 24] formed via the transient 5a-*ortho*-quinone methide intermediate **4** [25, 26] are quite well known as they have been a matter of extensive studies (Scheme 1). By contrast, research on tocopheramines had focused on interaction of tocopheramines with different radicals so far [27], but the minor oxidation products of α -tocopheramine have not yet been identified and characterized; and the corresponding analytical information, in particular NMR and MS data, is nearly completely lacking. Apart from a description of a nitroxide derivative which afforded a well-resolved EPR spectrum [28], and a UV/Vis-active by-product showing red color [29–31], none of the byproducts' structures has been proven and supported by detailed analytical data. However, the identification of the side products and their as standard

compounds would be an important prerequisite to usage as polymer stabilizer, and obviously for any biomedical application. It is reasonable to hypothesized that the byproducts formed would be N-oxidized compounds in general, which can be both N-oxygenated, e.g. hydroxylamine, nitroso or nitro derivatives, and N-coupled compounds, e.g. hydrazo, azo or azoxy derivatives, but none of them has ever been directly synthesized or characterized.

In this study, we report the synthesis of all monomeric, i.e. not N–N coupled, N-oxidized derivatives of α -tocopheramine, in an attempt to provide comprehensive analytical data and sufficient amounts in the 100 mg/1 g scale so that they could serve as standards, supporting identification of byproducts in unknown reaction mixtures by compound comparison.

Results and discussion

Upon oxidation of α -tocopheramine (**1**) in aqueous medium, formation of quinone **3** is consistently accompanied by byproducts, albeit only in the range of less than 1.5%. This explains on the one hand that these byproducts have so far eluded structure elucidation and, on the other hand, made it clear that this reaction would be inappropriate to provide larger amounts of these target compounds. α -Tocopherol (**2**)

Scheme 1

and α -tocopheramine (**1**) were considered as suitable starting materials for alternative syntheses, but considering that routes starting from the former would lead via the latter anyway left only the amine **1** as plausible option.

Organic methodology offers a whole wealth of procedures to oxidized aromatic amines to N-oxidized derivatives [32]. In the present case, the choice is more limited due to the labile, oxidant-sensitive nature of the substrate (tocopherol derivative!). Also, all methods which are not selective for one oxidation product, but would yield mixtures (mainly nitroso/nitro), were supposed to be excluded, as well as synthesis protocols operating in aqueous systems, which would yield quinone **3** as the major product. Another constraint was that the synthesis was supposed to follow green chemistry principles, which meant avoiding environmentally malign auxiliaries, solvents [33–35] and transition metal catalysts [36, 37], if possible. This avoidance was also plausible from the perspective that the substances were to be used in biological systems. These limitations narrowed down the number of promising protocols for the selective conversion of an aromatic amine into either a nitroso-, N-hydroxy- or nitrophenol, from initially more than 40 procedures to rather few based on hydrogen peroxide, peracids and their salts, e.g. peracetic acid, peroxomonosulfate, peroxodisulfate. Organic peroxides, e.g. *t*-butylhydroperoxide, *m*-chloroperbenzoic acid, yielded byproducts in the range of 20–30%, which were N–O coupling products with tocopheramine as well as tocopheramine dimers according to MS data, but were not investigated further.

Preliminary screening for the best systems showed the couple of glacial acetic acid and hydrogen peroxide [38] to give promising results. The fact that the ^{13}C NMR carboxyl resonance in the spectrum of the mixture gave a rather broad signal was an indication that peroxoacetic acid is involved in an equilibrium with acetic acid and may be the active oxidant. This was followed by optimization of the reaction conditions. Apparently, formation of *p*-tocopherylquinone (**3**) was hard to suppress and occurred already if traces of water were present, coming, for instance, from the hydrogen peroxide solution used. Hygroscopic additives, such as H_2SO_4 conc., were no remedy, but just increased the amount of (as yet unidentified) byproducts. In addition, it seemed hard to stop the oxidation at the nitroso stage and avoid “overoxidation” to the nitro compound, although lower temperatures significantly shifted the nitroso/nitro ratio in favor of the former.

The key was to conduct the reaction not with aqueous hydrogen peroxide, but to produce it in situ from suitable salts. Optimum results were provided with sodium percarbonate as H_2O_2 precursor, which in combination with glacial acetic acid and released from the solid complex by added sulfuric acid reacted quantitatively to a 2:3 molar mixture of 6-desoxy-6-nitroso- α -tocopherol (**6**) and

6-desoxy-6-nitro- α -tocopherol (**7**) at room temperature, notably without *p*-tocopherylquinone (**3**) being present. Solvents often used in vitamin E chemistry were tested, and both *n*-hexane and 1,4-dioxane gave similarly good results. However, the latter solvent was considered clearly superior due to the potential neurotoxicity of the former. The amount of H_2O_2 released can be exactly set by the amount of acid added and can be much better controlled than in the case of aqueous 30% H_2O_2 . Concentrated H_2SO_4 in 1,4-dioxane (1 M) gave the best results for that purpose. While sodium percarbonate is completely insoluble and can be added in excess, no free sulfuric acid is present in the system as it reacts immediately with the percarbonate releasing hydrogen peroxide. The presence of glacial acetic acid was imperative to obtain good yields: in its absence roughly 40% of byproducts were obtained, compared to near-quantitative product yields in its presence. This argued once more in favor of peroxoacetic acid as the actual oxidant which brings about the high selectivity, while hydrogen peroxide alone apparently was too harsh an oxidant [39].

Peroxomonosulfate and peroxodisulfate were too strong oxidants as well and gave only the nitro derivative in moderate yields, perborates gave yields below 45%, and the hydrogen peroxide urea complex caused extensive formation of unknown byproducts and chromophores. Another advantage of the percarbonate / glacial acetic acid / H_2SO_4 / dioxane system was that simply by adjusting the temperature the nitroso / nitro selectivity could be completely tuned (Scheme 2). At -5°C , only the nitroso derivative **6** was formed, even if the oxidant was present in moderate (three-fold) molar excess. At 50°C , only the nitro-compound **7** was obtained. Hence, no different setup for obtaining the nitro-derivative other than simply working at higher temperature than for the nitroso compound was required [40]. Both reactions provided virtually quantitative yields so that subsequent chromatographic separation was unnecessary. Also the work-up is strikingly easy: it is just done by removal of solids by filtration and solvent evaporation, which is a

Scheme 2

i = $\text{Na}_2\text{CO}_3 \cdot x\text{H}_2\text{O}_2$ (2.1 eq.), dioxane, HOAc glac., 1M H_2SO_4 conc. in dioxane, 45 min, -5°C , quant.
 ii = $\text{Na}_2\text{CO}_3 \cdot x\text{H}_2\text{O}_2$ (5.0 eq.), dioxane/HOAc glac., 1M H_2SO_4 conc. in dioxane, 5 min, 50°C , quant.
 iii = HOAc glac., 1 drop HCl (g) in MeOH, Mg pulv., 30 min, 65°C , quant.

distinct plus if the standard compounds are to be synthesized in labs not fully equipped for synthetic organic chemistry operations.

The best access to the *N*-hydroxy- α -tocopheramine (**8**) compound seemed to be the reduction of either nitro or nitroso derivative, as oxidative reactions starting from the amine would always be threatened by overoxidation to the nitroso or nitro compounds. The reduction of nitro-derivative **7** by SmI_2 gave an excellent 88% yield with 12% of amine **1** as the side product. However, the use of the toxic transition metal catalyst did not seem optimal to us. The alternative, the conventional reduction method with $\text{Zn}/\text{aq. NH}_4\text{Cl}$, provided initially fair yields of about 60% which were further improved to 74% of **8** by working in glacial acetic acid, although still being accompanied by significant amine formation (26% of **1**). Exchanging Zn for Mg as the reductant, adding a drop of gaseous HCl in MeOH before the metal addition and working at -20°C throughout, was the key in avoiding overreduction to the amine and getting quantitative yields of hydroxylamine. The practical advantage of the approach is that it can be conducted as one-pot procedure starting from amine **1** via nitro derivative **7** (Scheme 2).

Table 1 provides the NMR data of the N-oxidized compounds **6–8** in comparison to the starting amine **1**. In ^1H NMR the compounds can be distinguished by the effect of the 5-substituents of the protons of the aromatic methyl groups, which—by analogy to tocopherols—is most pronounced for C-5a, followed by C-7a and C-8b. In ^{13}C NMR, the nitroso derivative **6** is distinguished by a large C-6 downfield shift of more than 30 ppm, compared to 15 ppm for

the nitro-derivative **7** and 11 ppm for the hydroxylamine **8**. For the nitroso and nitro derivatives, the two *ortho*-positions (C-5 and C-7) as well as the *para*-position (C-8a) are affected by downfield shifts, which are not seen for the hydroxylamino derivative, of which the shifts were closer to the starting amine **1**.

All three derivatives were completely stable when stored under an inert gas in the dark; no chemical changes were detectable after storage over more than 8 months under these conditions. The same is true for degassed (oxygen-free) solutions in *n*-hexane or 1,4-dioxane. All three derivatives **6–8** are tolerant towards acids (1 M H_2SO_4 , glacial acetic acid, phosphate buffer pH 1 in aqueous methanol), and the nitro-derivative **7** and hydroxylamine derivative **8** also towards bases and alkali (triethylamine, 1 M NaOH). The nitroso-derivative **6**, when treated with alkali (1 M NaOH in aqueous methanol), provided a single compound which was identified as the spiro-dimer of α -tocopherol (**5**), which is usually formed by dimerization of the *ortho*-quinone methide **4** derived from α -tocopherol, a very common intermediate in vitamin E chemistry, see Scheme 1. It was logical to assume that this *ortho*-quinone methide intermediate would also be involved in the present case, albeit coming from a different precursor. When repeating the reaction with the ^{15}N -labeled model compound 6-(^{15}N -amino)-2,2,5,7,8-pentamethylchroman (**1a**) at r.t. in the NMR tube, an intermediate at -42.6 ppm was observed, which changed into a stable shift at -276.2 ppm. While the first shift is in the typical range of oximes, the second value originates from free hydroxylamine, which was unambiguously proven by

Table 1 ^1H and ^{13}C chemical shifts (δ/ppm) of α -tocopheramine (**1**) and its N-oxidized derivatives **6–8** in CDCl_3 as the solvent (25°C)

Nucleus/position ^a	α -Tocopheramine (1)	Nitro derivative 7	Nitroso derivative 6	Hydroxylamine derivative 8
H-2a	1.38 (s, 3H)	1.37 (s, 3H)	1.38 (s, 3H)	1.38 (s, 3H)
H-3	1.73 (m, 2H)	1.83 (m, 2H)	1.81 (m, 2H)	1.83 (m, 2H)
H-4	2.66 (“t”, 2H)	2.62 (“t”, 2H)	2.64 (“t”, 2H)	2.64 (“t”, 2H)
H-5a, 7a, 8b	2.16, 2.13, 2.09 (3×s, 3×3H)	2.26, 2.13, 2.12 (3×s, 3×3H)	2.18, 2.16, 2.11 (3xs, 3×3H)	2.14, 12.12, 2.09 (3×s, 3×3H)
N–H	4.9 (s, br, 1H)	–	–	4.9 (s, br, 2H)
C-2	74.3	74.6	74.4	74.3
C-2a	28.0	27.9	28.0	28.0
C-3	32.7	32.5	32.6	32.5
C-4	22.7	22.0	22.1	22.4
C-5a, 7a, 8b	13.6, 12.6, 11.9	15.3, 14.6, 12.3	17.4, 15.1, 12.2	13.8, 12.2, 12.0
C-4a	122.3	118.9	120.7	123.0
C-5	117.1	125.5	122.4	116.4
C-6	134.7	145.9	168.1	140.3
C-7	117.8	126.0	125.2	116.9
C-8	120.6	125.1	129.4	121.7
C-8a	145.0	151.9	148.4	144.4

^aAtom numbering see Scheme 1

spiking with the authentic compound. The nitroso-compound **6**, upon alkali treatment, would thus undergo a tautomeric [1, 5]-sigmatropic proton shift involving the C-5a methyl group, leading to a transient *ortho*-quinone methide oxime (**9**), in fact the oxime of *ortho*-quinone methide **4**, see Scheme 3. The special activation of the C-5a methyl group and its preference over the alternative *ortho*-methyl group at C-7a is well known in vitamin E chemistry and has been accounted for by the theory of strain-induced bond localization (SIBL) [26, 41]. The *ortho*-quinone methide involving C-5a experiences less strain than the alternative C-7a-*ortho*-quinone methide and is just energetically favored. The same would be true also for their oximes; note that a distinction between *E*- or *Z*-configuration of the oxime intermediate cannot be made based on the present data. In the presence of water, the oxime releases hydroxylamine to form **4** which immediately dimerizes to the spiro-dimer **5** (see above). In α -tocopherol chemistry, this dimerization is an astonishingly clean process which gives the neat spirodimer in quantitative yield, and the same course was observed in the present case, making alkali treatment of the nitroso-compound **6** an elegant route to spiro-dimer **5** starting from tocopheramine material.

To provide additional structural information about the N-oxidized derivatives, the above syntheses were repeated with the model compound **1a** carrying a methyl group instead of the isoprenoid side chain and an ^{15}N -isotopic label, which was available from previous work [42]. **1a** is the amino analogue of the well-known truncated α -tocopherol model compound 2,2,5,7,8-pentamethylchroman-6-ol [43]. The N-oxidized derivatives (nitroso: **6a**, nitro: **7a**, hydroxylamino: **8a**) showed characteristic ^{15}N NMR shifts, all in the expectable ranges, and thus confirmed the ^1H and ^{13}C NMR data, as shown in Scheme 4. Note the characteristic, large positive ^{15}N shift of the nitroso derivative. To rationalize

reactivity and NMR shift effects of the nitrogen substituents it is helpful to think of nitroso, nitro, and hydroxylamino groups as hetero-analogues of carbonyl ($\text{C}=\text{O}$), carboxyl (COOH), and hydroxymethyl (CH_2OH) moieties, respectively.

Conclusion

The optimized synthetic procedures provided access to the monomeric N-oxidized derivatives **6–8** of α -tocopheramine (**1**) in quantitative yields, without the need for chromatographic purification and avoiding solvents and auxiliaries that would contradict green chemistry principles. Compounds **6–8** can alternatively be perceived as the 6-desoxy-6-nitroso, 6-desoxy-6-nitro, and 6-desoxy-6-hydroxylamino derivatives, respectively, of α -tocopherol (**2**). With these compounds, standards are accessible that will allow easy and fast identification of the byproducts in oxidation reactions of α -tocopheramine, even if they are formed only in trace amounts. While the nitroso, nitro, and hydroxylamine derivatives are the dominant byproducts of oxidations carried out in aqueous media, such as under physiological conditions, some dimers of α -tocopheramine, probably of the hydrazo, azo or azoxy type, dominate under non-aqueous conditions, e.g. when tocopheramine is applied as a polymer (melt) stabilizer. The structural identification and synthesis of these dimeric N-oxidation products will be the topic of an upcoming account.

Scheme 3

Scheme 4

Experimental

All chemicals were commercial products, of the highest purity available and used without further purification. HPLC-grade solvents were used for all extractions and workup procedures. Bidistilled water was used for all aqueous extractions and for all aqueous solutions. 1,4-Dioxane, *n*-heptane, ethyl acetate, and toluene used in chromatography were distilled before use. α -Tocopheramine (**1**) was of the [*R,R,R*]-type, maintenance of stereochemical integrity over the reactions performed was not further checked, however.

All reactions involving non-aqueous conditions were conducted in oven-dried (140 °C, overnight) glassware under an argon atmosphere. TLC was performed using Merck silica gel 60 F₂₅₄ pre-coated plates, and flash chromatography on Baker silica gel (40 μ m particle size). All products were purified to homogeneity by TLC / GC analysis; yields refer to isolated, pure products with satisfying elemental analysis data (± 0.2). Elemental analyses were performed at the Microanalytical Laboratory of the University of Vienna. Melting points, determined on a Kofler-type micro hot stage with Reichert-Biovar microscope, are corrected.

¹H NMR spectra were recorded at 300.13 MHz for ¹H and at 75.47 MHz for ¹³C NMR in CDCl₃ if not otherwise stated. Chemical shifts, relative to TMS as internal standard, are given in δ values, coupling constants in Hz. ¹³C peaks were assigned by means of APT, HMQC, and HMBC spectra, “d.i.” denoting peaks with double intensity.

The nomenclature and atom numbering of tocopherols and chromanols as recommended by IUPAC was used throughout [44, 45]. ¹H and ¹³C NMR resonances of the isoprenoid side chain of tocopherols are only insignificantly influenced ($\Delta < 0.05$ ppm) by modifications of the chroman ring [46, 47], and are thus listed only once: 19.7 (C-4a'), 19.8 (C-8a'), 21.2 (C-2'), 22.7 (C-13'), 22.8 (C-12a'), 24.6 (C-6'), 24.8 (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'), 37.5 (C-3'), 39.3 (C-11'), 39.9 (C-1') ppm. Analytical data for α -tocopheramine (**1**) agreed with the literature: [2] for methods apart from NMR, and [7] for NMR data, and so did the data for the ¹⁵N-labeled model compound **1a** [41].

(R)-2-[(4*R*,8*R*)-4,8,12-Trimethyltridecyl]-2,5,7,8-tetramethyl-6-nitroso-chroman (6-desoxy-6-nitroso- α -tocopherol, **6, C₂₉H₄₉NO₂)** α -Tocopheramine (**1**, 0.43 g, 1.00 mmol) was dissolved in freshly distilled 1,4-dioxane (25 cm³). Glacial acetic acid (15 cm³) and freshly powdered sodium percarbonate (0.33 g, 2.10 mmol) were added and the mixture cooled to -5 °C (ice/NaCl bath) under stirring. A solution of concentrated H₂SO₄ in 1,4-dioxane (1 M, 2.15 cm³) was slowly added (ca. 15 min) and the mixture stirred for

another 30 min. Freshly powdered, anhydrous sodium carbonate (ca. 1.00 g) was added, the mixture warmed to r.t. under stirring, and the solids were removed by filtration. Evaporation of the solvent in vacuo afforded 0.41 g (93%) of **6**. *R_f* (toluene)=0.72; ¹H NMR and ¹³C NMR, see Table 1; EI-MS (70 eV): *m/z* (%) = 445 (MH⁺, 30), 444 (55), 218 (25), 177 (100), 57 (10), 43 (20); [α]_D²⁰ = +34.2° cm² g⁻¹ (*c* = 1, ethanol).

(R)-2-[(4*R*,8*R*)-4,8,12-Trimethyltridecyl]-2,5,7,8-tetramethyl-6-nitro-chroman (6-desoxy-6-nitro- α -tocopherol, **7, C₂₉H₄₉NO₃)** α -Tocopheramine (**1**, 0.43 g, 1.0 mmol) was dissolved in freshly distilled 1,4-dioxane (25 cm³). Glacial acetic acid (15 cm³) and freshly powdered sodium percarbonate (0.78 g, 5.00 mmol) was added and the mixture heated to 50 °C under stirring. A solution of concentrated H₂SO₄ in 1,4-dioxane (1 M, 10 cm³) was added (ca. 1 min) and the mixture stirred for another 5 min. Freshly powdered, anhydrous sodium carbonate (ca. 1.00 g) was added, the mixture cooled to r.t. under stirring, and the solids were removed by filtration. Evaporation of the solvent in vacuo afforded 0.45 g (98%) of **7**. *R_f* (toluene)=0.64; ¹H NMR and ¹³C NMR, see Table 1; EI-MS (70 eV): *m/z* (%) = 461 (MH⁺, 65), 460 (25), 235 (20), 194 (100), 57 (15), 46 (10), 43 (20); [α]_D²⁰ = +7.4° cm² g⁻¹ (*c* = 1, ethanol).

(R)-2-[(4*R*,8*R*)-4,8,12-Trimethyltridecyl]-2,5,7,8-tetramethyl-6-chroman-yl-*N*-hydroxylamine (6-desoxy-6-hydroxylamino- α -tocopherol, **8, C₂₉H₅₁NO₂)** Magnesium powder (0.24 g, 10 mmol) was added to the above obtained solution (in 1,4-dioxane and glacial acetic) of nitro-derivative **7** at r.t. One drop of a solution of gaseous HCl in methanol (3 M) was added and the mixture heated to 65 °C for 30 min under stirring. After cooling to r.t., freshly powdered sodium carbonate (ca. 1 g) was added, the mixture stirred for 15 min, left standing for 5 min and filtered to remove the solids. Evaporation of the solvent in vacuo afforded 0.39 g (88%) of **8**. *R_f* (toluene)=0.48; ¹H NMR and ¹³C NMR, see Table 1; EI-MS (70 eV): *m/z* (%) = 447 (MH⁺, 15), 446 (75), 221 (45), 180 (100), 57 (10), 43 (15); [α]_D²⁰ = +16.5° cm² g⁻¹ (*c* = 1, ethanol).

2,2,5,7,8-Pentamethyl-6-nitrosochroman-¹⁵N (6a, C₁₄H₁₉NO₂) The procedure follows the above protocol for the preparation of **6**, employing model compound **1a** instead of α -tocopheramine (**1**). Evaporation of the solvent in vacuo afforded a solid that was crystallized twice from *n*-heptane to afford 0.16 g (67%) of **6a**. M.p.: 89–91 °C; *R_f* (*n*-heptane / ethyl acetate, *v/v* = 10:1) = 0.72; ¹H NMR: δ = 2.64 (2H, t, ³*J* = 7.0 Hz, 4-CH₂), 2.17 (s, 3H, 5a-CH₃), 2.16 (s, 3H, 7a-CH₃), 2.12 (s, 3H, 8b-CH₃) 1.82 (t, 2H, ³*J* = 6.80 Hz, 3-CH₂), 1.33 (s, 6H, 2a-CH₃) ppm; ¹³C NMR: δ = 166.4

(C-6, d, C-6, $J_{\text{C,N}} = 18.4$ Hz), 148.2 (C-8a), 126.2 (C-7), 125.8 (C-5), 122.3.0 (C-8), 120.4 (C-4a), 74.2 (C-2), 32.5 (C-3), 27.3 (C-2a), 21.4 (C-4), 17.3 (C-7a), 15.4 (C-5a), 12.3 (C-8b) ppm; ^{15}N NMR: $\delta + 508.4$ ppm; EI-MS (70 eV): m/z (%) = 235 (MH^+ , 15), 234 (53), 217 (40), 203 (42%), 180 (16), 179 (100), 162 (14), 161 (30), 91 (10), 77 (10).

2,2,5,7,8-Pentamethyl-6-nitrochroman- ^{15}N (7a, $\text{C}_{14}\text{H}_{19}\text{NO}_3$) The procedure follows the above protocol for the preparation of **7**, applying model compound **1a** instead of α -tocopheramine (**1**). Evaporation of the solvent in vacuo and flash chromatography (*n*-heptane / ethyl acetate, $v/v = 10:1$) afforded 0.22 g (88%) of **7**. M.p.: 143–144 °C; R_f (*n*-heptane / ethyl acetate, $v/v = 10:1$) = 0.61; ^1H NMR: $\delta = 2.62$ (2H, t, $^3J = 6.80$ Hz, 4- CH_2), 2.14 (s, 3H, 5a- CH_3), 2.12 (s, 3H, 7a- CH_3), 2.11 (s, 3H, 8b- CH_3) 1.82 (t, 2H, $^3J = 6.80$ Hz, 3- CH_2), 1.32 (s, 6H, 2a- CH_3) ppm; ^{13}C NMR: $\delta = 152.8$ (C-8a), 146.6 (d, C-6, $J_{\text{C,N}} = 14.0$ Hz), 126.5 (C-7), 125.4 (C-5), 122.1 (C-8), 119.3 (C-4a), 74.4 (C-2), 32.5 (C-3), 27.1 (C-2a), 21.2 (C-4), 14.9 (C-7a), 14.1 (C-5a), 12.1 (C-8b) ppm; ^{15}N NMR: $\delta = -10.1$ ppm; EI-MS (70 eV): m/z (%) = 251 (MH^+ , 10), 250 (64), 233 (30), 196 (11), 195 (100), 178 (22), 177 (23), 91 (14), 77 (10).

2,2,5,7,8-Pentamethyl-6-chromanyl-N-hydroxylamine- ^{15}N (8a, $\text{C}_{14}\text{H}_{21}\text{NO}_2$) The procedure follows the above protocol for the preparation of **8**, employing model compound **7a** instead of the nitro-tocopherol **7**. Evaporation of the solvent in vacuo and flash chromatography (*n*-heptane / ethyl acetate, $v/v = 10:1$) afforded 0.19 g (81%) of **8**. M.p.: 72–74 °C; R_f (*n*-heptane / ethyl acetate, $v/v = 10:1$) = 0.49; ^1H NMR: $\delta = 2.61$ (2H, t, $^3J = 6.8$ Hz, 4- CH_2), 2.13 (s, 3H, 5a- CH_3), 2.12 (s, 3H, 7a- CH_3), 2.10 (s, 3H, 8b- CH_3) 1.82 (t, 2H, $^3J = 6.8$ Hz, 3- CH_2), 1.33 (s, 6H, 2a- CH_3) ppm; ^{13}C NMR: δ 145.4 (C-8a), 140.8 (d, C-6, $J_{\text{C,N}} = 6.6$ Hz), 122.8 (C-4a), 121.8 (C-8), 117.1 (C-5), 116.5 (C-7), 74.4 (C-2), 32.5 (C-3), 27.4 (C-2a), 21.5 (C-4), 13.5 (C-7a), 12.5 (C-5a), 12.0 (C-8b) ppm; ^{15}N NMR: $\delta = -251.3$ ppm; EI-MS (70 eV): m/z (%) = 237 (MH^+ , 85), 236 (22), 219 (23), 203 (40%), 182 (16), 181 (100), 179 (22%), 164 (30), 163 (12), 91 (10), 77 (10).

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References

- Smith LI, Renfrow WB, Opie JW (1942) J Am Chem Soc 64:1082
- Mayer H, Isler O (1971) Tocopheramines and tocopherthiols. In: Colowick SP, Kaplan NO (eds) Methods in enzymology, vol 18, part C. Academic Press, New York, pp 275–334
- Blomstrand R, Forsgren L (1968) Int J Vit Res 38:328
- Bieri JG, Prival EL (1967) Biochem 6:2153
- Itoh S, Nagaoka S, Mukai K, Ikesu S, Kaneko Y (1994) Lipids 29:799
- Preedy VR, Watson RR (2007) Encyclopedia of vitamin E. CABI Publishing, Oxford
- Mazzini F, Netscher T, Salvadori P (2009) Eur J Org Chem 13:2063
- Schwieter U, Schlegel W, Tamm R (1969) Non-toxic antioxidants, based on chromane derivatives. US Pat. 3458637. Chem Abstr 69:21909
- Søndergaard E, Dam H (1970) Z Ernähr Swiss 10:71
- Lambert KJ, Lal M (2002) Preparation of aminobenzopyran derivatives as surfactants. Patent WO 2002076937. Chem Abstr 137:263202
- Tokuwame M (1991) Synergistic antioxidant-heat stabilizer systems for polyolefins. JP Pat. 03043458. Chem Abstr 115:73015
- Tomic-Vatic A, Eytina J, Chapman J, Mahdavian E, Neuzil J, Salvatore BA (2005) Int J Cancer 117:188
- Gille L, Stamberger W, Patel A, Böhmendorfer S, Rosenau T (2012) Free Rad Biol Med 53:100
- Zingg JM (2007) Mini Rev Med Chem 7:545
- Neuzil J, Tomasetti M, Zhao Y, Dong LF, Birringer M, Wang XF, Low P, Wu K, Salvatore BA, Ralph SJ (2007) Mol Pharmacol 71:1185
- Gruber J, Staniek K, Krewenka C, Moldzio R, Patel A, Böhmendorfer S, Rosenau T, Gille L (2014) Bioorg Med Chem 22:684
- Tanito M, Yoshida Y, Kaidzu S, Chen ZH, Cynshi O, Jishage KI, Niki E, Ohira A (2007) Investig Ophthaomol Vis Sci 48:396
- Bieri JG, Mason KE (1968) J Nutr 96:192
- Rosenau T, Potthast A, Adorjan I, Hofinger A, Sixta H, Firgo H, Kosma P (2002) Cellulose 9:283
- Rosenau T, Potthast A, Kosma P, Chen CL, Gratzl JS (1999) J Org Chem 64:2166
- Rüegg R, Mayer H, Schudel P, Schwietter U, Tamm R, Isler R (1967) Wiss. Veröff. Dtsch Ges. Ernähr. 16:22
- Machlin LJ (1980) Vitamin E: a comprehensive treatise. Marcel Dekker Inc, New York
- Schudel P, Mayer H, Metzger J, Rüegg R, Isler O (1963) Helv Chim Acta 46:636
- Schröder H, Netscher T (2001) Magn Reson Chem 39:701
- Rosenau T, Potthast A, Elder T, Kosma P (2002) Org Lett 4:4285
- Rosenau T, Böhmendorfer S (2009) ortho-Quinone methides in tocopherol chemistry. In: Rokita S (ed) Wiley series on reactive intermediates in chemistry and biology, vol 1. Wiley, New York, p 163

27. Bamonti L, Hosoya T, Pirker K, Böhmendorfer S, Mazzini F, Galli F, Netscher T, Rosenau T, Gille L (2013) *Bioorg Med Chem* 21:5039
28. Murphy PA, Lin JS, Olcott HS, Windle JJ (1976) *Lipids* 11:296
29. Boguth W, Hackel R (1965) *Hoppe-Seyler Z Physiol Chem* 342:172
30. Igarashi O (1977) *J Nutr Sci Vitaminol* 23:169
31. Quaife ML (1948) *J Biol Chem* 175:605
32. Fieser M, Fieser L (eds) (2007) *Reagents for organic synthesis*. John Wiley and Sons Inc, Hoboken
33. Das SS, Nath U, Deb D, Das PJ (2004) *Synth Commun* 34:2359
34. Gowenlock BG, Richter-Addo GB (2004) *Chem Rev* 104:3315
35. Vančik H (2013) *Aromatic C-nitroso Compounds*. Springer, Dordrecht
36. Nguyen THL, Gigant N, Delarue-Cochin S, Joseph D (2016) *J Org Chem* 81:1850
37. Seok WK, Meyer TJ (2004) *Inorg Chem* 43:5205
38. Holmes RR, Bayer RP (1960) *J Am Chem Soc* 82:3454
39. Gorrod JW (1968) *Tetrahedron Lett* 9:6155
40. Gilbert EE, Leccacorvi JR (1976) *Propellant Explos Pyrotech* 1:89
41. Rosenau T, Ebner G, Stanger A, Perl S, Nuri L (2005) *Chem Eur J* 11:280
42. Böhmendorfer S, Gille L, Rosenau T (2010) *Lett Org Chem* 7:335
43. Rosenau T (2007) 2,2,5,7,8-Pentamethylchroman-6-ol (PMC) and related model compounds. In: Preedy VR, Watson RR (eds) *Encyclopedia of vitamin E*. CABI Publishing, Oxford, p 21
44. IUPAC-IUB Commission on Biochemical Nomenclature (CBN) (1982) *Eur J Biochem* 123:473
45. IUPAC-IUB Commission on Biochemical Nomenclature (CBN) (1974) *Arch Biochim Biophys* 165(1):6–8
46. Urano S, Hattori Y, Yamanoi S, Matsuo M (1980) *Chem Pharm Bull* 28:1992
47. Brownstein S, Ingold KU (1989) *J Org Chem* 54:560

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