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Antioxidant dehydrotocopherols as a new chemical character of *Stemona* species

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Abstract

From the roots of various *Stemona* species four new dehydrotocopherols (chromenols) were isolated and their structures and stereochemistry elucidated by spectroscopic methods. The double bond between C-3 and C-4 proved to be a typical chemical character of the genus found in most of the species. Various C-methylations of the aromatic ring reflect differences in methyltransferase activities and agreed with the current species delimitations showing an exclusive accumulation of dehydro-δ-tocopherol for the *Stemona tuberosa* group, whereas different provenances of *Stemona curtisii* were characterized by dehydro-γ-tocopherol accompanied by small amounts of dehydro-α-tocopherol. From *Stemona collinsae* all four tocopherols were isolated with a clear preponderance of dehydro-δ-tocopherol accompanied by smaller amounts of the rare dehydro-β-tocopherol. *Stemona burkillii* and a group of unidentified species showed a weak accumulation trend towards dehydro-α-tocopherol, whereas *Stemona cochinchinensis* and especially *Stemona kerrii* clearly differed by a preponderance of chromanol derivatives. In *Stemona* cf. *pierrei* no tocopherols could be detected at all. Based on TLC tests and microplate assays with the free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH·) the antioxidant capacities of all chromenol derivatives were comparable with that of α-tocopherol showing no significant differences among each other, except for a more rapid kinetic behaviour of the 5,7,8-methylated dehydro-α-tocopherol.

Keywords: *Stemona tuberosa*; *Stemona collinsae*; *Stemona curtisii*; *Stemona cochinchinensis*; *Stemona kerrii*; *Stemona burkillii*; *Stemona pierrei*; Stemonaceae; Tocopherols; Dehydrotocopherols; Antioxidant; Radical scavenger; Chemosystematics

1. Introduction

Tocopherols have been found in nearly all photosynthetic organisms (Lichtenthaler, 1968) and constitute the vitamin E group together with the related tocotrienols, esters and derivatives. Structurally they are lipid-soluble chromanols linked to a prenyl side chain which is either saturated in the tocopherols or unsaturated in the tocotrienols. Because of their important role in nutrition and health (Eitenmiller, 1997; Grusak and DellaPenna, 1999), particularly due to their antioxidant activities as free radical scavengers (Niki and Noguchi, 2004), a considerable number of publications already exists mainly focusing on function in biological membranes, isolation from plant extracts, and synthesis (for review see Munné-Bosch and Alegre, 2002). Besides the degree of saturation of their prenyl side chain, various C-methylations of the aromatic ring also contribute to

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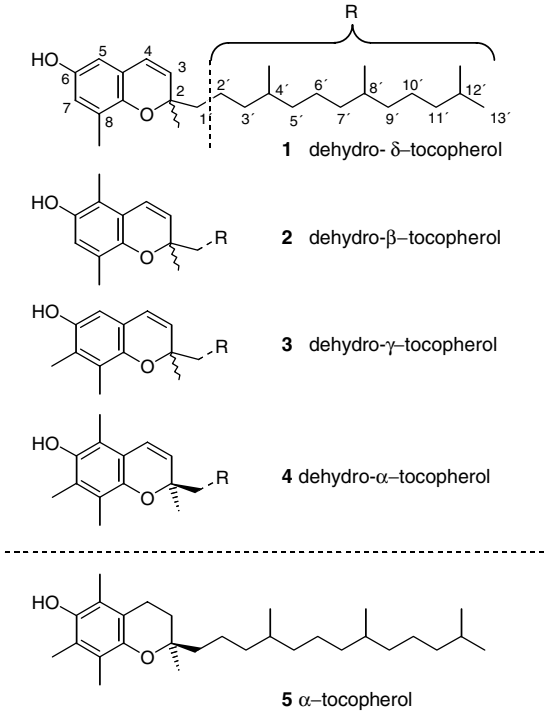
structural variety leading, e.g., to the formation of the four types α-, β-, γ- and δ-tocopherol. However, common to all naturally occurring derivatives isolated so far was a chromanol basic skeleton. To date, corresponding chromenols are known only as synthetic products and the NMR data presented are sparse. Dehydro-α-tocopherol was reported in several papers (McHale and Green, 1965; Asgill et al., 1978; Omura, 1989; Lei and Atkinson, 2000) from which only the latter two listed NMR data without assignments. Dehydro-β-tocopherol was mentioned only once, however, no spectroscopic data were given at all (Asgill et al., 1978). In the case of dehydro-γ-tocopherol only its 6-methoxy derivative has been described by Stocker et al. (1994), whereas for dehydro-δ-tocopherol no report could be found. Hence, our present finding of chromenol derivatives in different *Stemona* species is the first report on naturally occurring 3,4-dehydrotocopherols. In the present paper we report the isolation and structure elucidation of the four 3,4-dehydrotocopherols **1**–**4** by spectroscopic methods.

Whereas the antioxidant activity of tocopherols and tocotrienols with a chromanol skeleton is well described in literature (Munné-Bosch and Alegre, 2002), nothing is known about related chromenols containing an extended chromophoric system. Thus, we compared the antioxidant activities of four naturally occurring 3,4-dehydrotocopherols (**1**–**4**) with that of a commercial α-tocopherol (**5**) using the free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH·) as indicator for antiradical capacity (Kurechi et al., 1980; Brand-Williams et al., 1995; Matsukawa et al., 1997; Espín et al., 2000).



2. Results and discussion

2.1. Structure elucidation

From the methanolic crude extracts of the air-dried tuberous roots of various *Stemona* species we previously described a series of family-specific alkaloids (Brem et al., 2002; Kaltenegger et al., 2003) and stilbenoids (Pacher et al., 2002; Kostecki et al., 2004). In addition, comparative HPLC–UV analyses exhibited a series of unpolar compounds with characteristic UV maxima in MeOH in the regions at 332–338, 268–280 and 232 nm (Fig. 1). For their isolation and structure elucidation the more lipophilic column fractions of *Stemona collinsae* Craib, *Stemona tuberosa* Lour., and *Stemona curtisii* Hook.f. were used and eluted with 10–25% EtOAc in hexane. Preparative MPLC of the combined fractions afforded a mixture which was further separated by TLC. The IR spectra of the isolated compounds were mainly characterized by strong signals at 1461–1463 cm^{-1} and a typical band at 3616–3626 cm^{-1} (CCl_4), indicative for a phenolic OH group (see Section 3).

The ^1H NMR spectrum of compound **1**, isolated from *S. collinsae* and *S. tuberosa*, was characterized by an olefinic or aromatic AB system at $\delta = 6.24$ and 5.59 ppm ($J = 9.7$ Hz). Additionally, resonances of a D_2O exchangeable phenolic OH at 4.32 ppm (broad *s*) and an aromatic methyl group at 2.13 ppm together with a second aromatic AB system at $\delta = 6.47$ and 6.32 ppm ($J = 2.9$ Hz, *meta* coupling) were characteristic substituents of an aromatic ring system. All other protons were aliphatic and consisted of a methyl singlet at 1.35 ppm and a saturated terpenoid side chain characterized by four methyl dublets, a multiplet at 1.62 ppm (2H), and a nonett at 1.53 ppm (1H) with two methyl groups and one methylene group as neighbours. The remaining aliphatic protons (18H) resonated in the range of 1.0–1.4 ppm. Additionally, the ^{13}C NMR spectrum showed five quaternary atoms, four of them in the aromatic region and one also at relatively low field but not aromatic (78 ppm). The number of quaternary C, CH, CH_2, and CH_3 groups (^{13}C NMR) plus one OH (^1H NMR) and a further ether oxygen agreed with a molecular formula of $C_{27}H_{44}O_2$ derived from HREIMS. The parent peak in the EIMS was at $m/z = 175$ after loss of the C_{16} side chain. All these data for compound **1** were in favour of a 3,4-dehydro-δ-tocopherol with a maximally conjugated aromatic chromene system. A thorough 2D NMR analysis (H/H COSY, NOESY, HMQC, and HMBC) confirmed this structure. NOESY contacts proved the aromatic substitution pattern and HMBC long range connectivities allowed a complete assignment of all ^{13}C resonances (see Section 3).

However, two points of importance have still to be discussed: the first was a contamination with the corresponding chromanol which could not be removed chro-

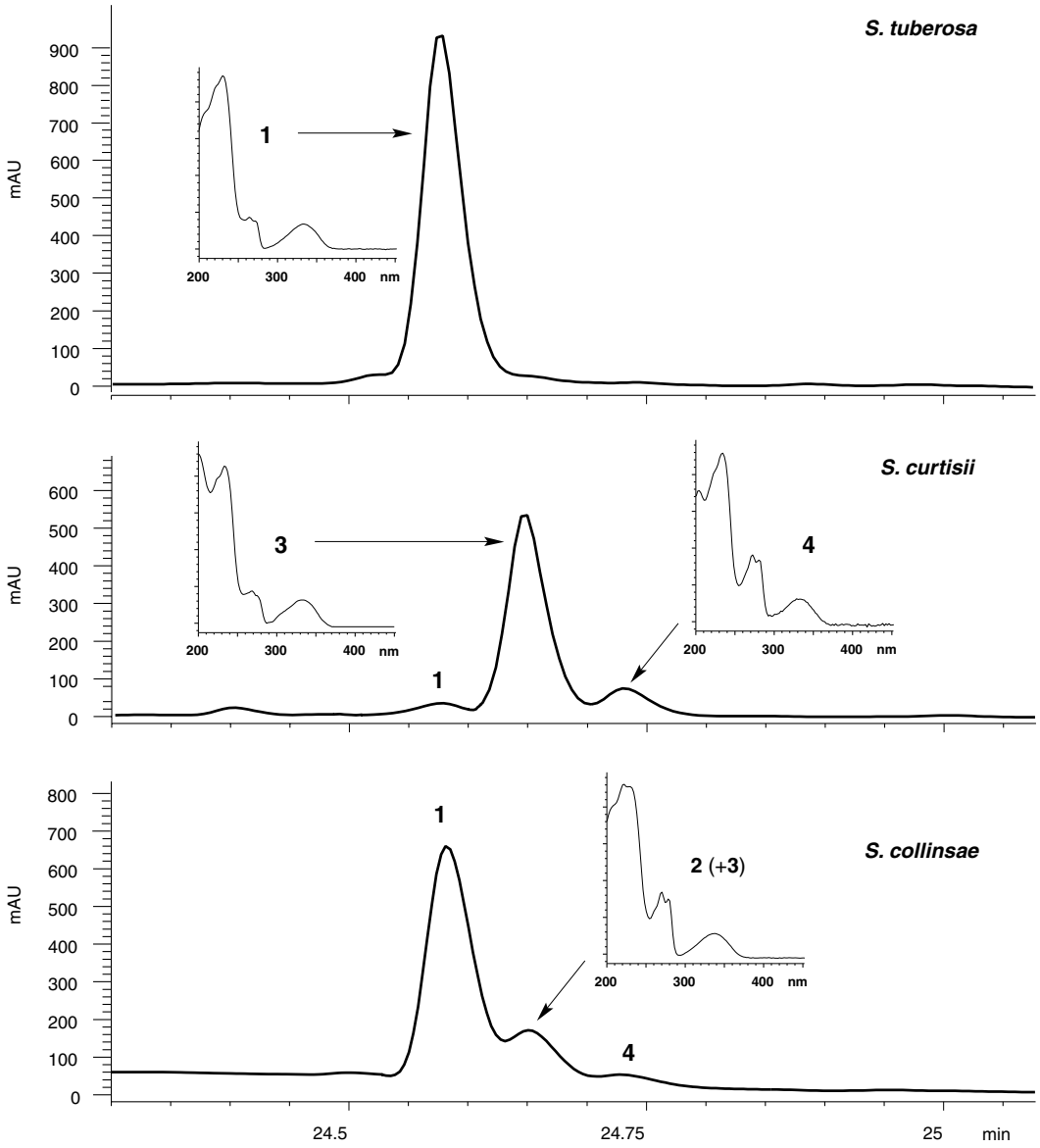


Fig. 1. HPLC comparison of the unpolar region of the crude extracts of *S. tuberosa* (HG 851), *S. curtisii* (HG 917), and *S. collinsae* (HG 840) showing the different dehydrotocopherol compositions and characteristic UV spectra of the derivatives **1**–**4** (see Section 3). UV diode array detection at 230 nm.

matographically, whereas the second point concerns the stereochemistry. In compound **1**, only traces of contamination with the known δ-tocopherol could be detected. However, the saturated second ring in the structure was unambiguously indicated by the two methylene groups of the chromanol system. They showed characteristic resonances for a broad *t* at $\delta = 2.68$ ppm for 4-H_2 and a multiplet for two slightly separated diastereotopic protons centered at 1.73 ppm for 3-H_2. According to the integrations in the ^{1}H NMR spectrum, compound **1** from *S. collinsae* was contaminated with 4%, and in case of *S. tuberosa* with only 1% δ-tocopherol. With respect to the stereochemistry of **1**, it turned out that a diastereomeric mixture of a ratio ~60:40% has been isolated. This was surprising with respect to the proposed biosynthetic route of γ-tocopherol to only one diastereomeric form catalyzed by tocopherol cyclase (Stocker et al., 1994). However, the diastereomeric mixture of dehydro-δ-tocopherol (**1**) was indicated unambiguously by the analysis of several twin signals in the ^{13}C NMR spectrum in $CDCl_3$, showing diastereomeric shift differences up to 0.05 ppm (Fig. 2). The experimental $\Delta(\delta)$ ppm values were for C-3 = −0.02, C-1′ = +0.04, C-2′ = +0.01, and 4′-CH_3 = +0.05. The signs were determined by subtracting the shift values of the minor diastereomer from the dominating one (Table 1). Since the systematic measurements of Brownstein et al. (1989) for the diastereomeric α-tocopherols were performed in acetone-d_6 we

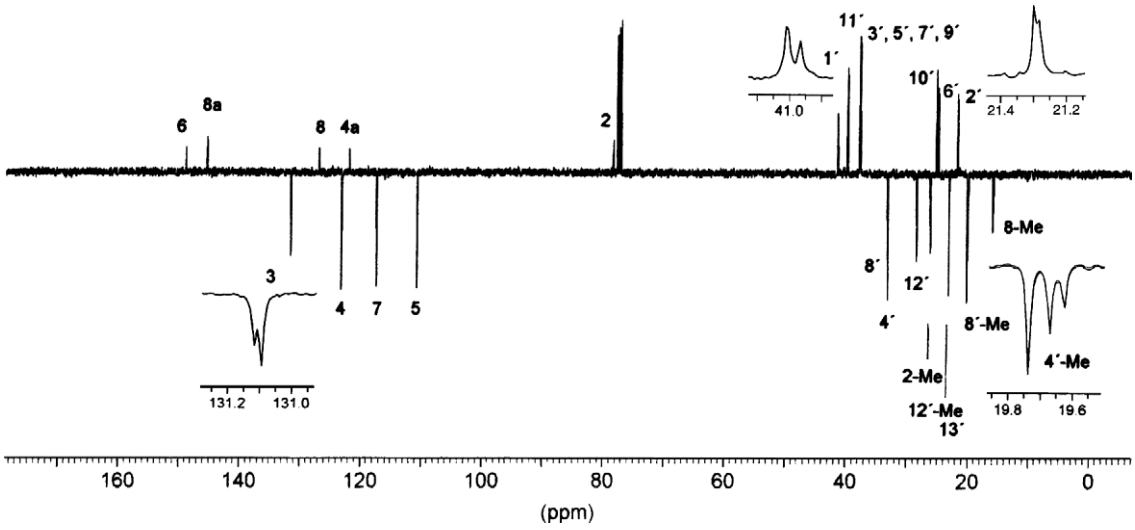


Fig. 2. ^{13}C NMR spectrum of dehydro-δ-tocopherol (**1**) in $CDCl_3$. The regions at 131.1 (C-3), 41.0 (C-1′), 21.3 (C-2′), and 19.7 ppm (8′-Me and 4′-Me) are shown in details as inserts. The larger peaks of the split signals for C-3, C-1′, C-2′, and 4′-Me correspond to the (*S*,*R*,*R*) diastereomer, the smaller ones to (*R*,*R*,*R*).

also measured the ^{13}C NMR spectrum of **1** in this solvent, which gave the following diastereomeric shift differences: C-3 = −0.03, C-1′ = +0.05, C-2′ = +0.04, and 4′-CH_3 = +0.04 (see Section 3). Careful comparison of our ^{13}C NMR shift data with those of Brownstein et al. (1989) revealed that our data could be easily interpreted by assuming a diastereomeric mixture of ~60% (*S*,*R*,*R*) configuration with ~40% of the (*R*,*R*,*R*) diastereomer under the reasonable assumption that the diastereomeric shift differences Δ(δ) ppm of the side chain carbon atoms should not be significantly influenced by the different aromatic systems. The fact that all three

Table 1
^{13}C NMR data of dehydrotocopherols **1**–**4** ($CDCl_3$, δ/ppm)

	1 (δ) *S*,*R*,*R*/*R*,*R*,*R* = 60/40%	**2** (β) *R*,*R*,*R*/*S*,*R*,*R* = 90/10%	**3** (γ) *S*,*R*,*R*/*R*,*R*,*R* = 60/40%	**4** (α) *R*,*R*,*R*
2	77.97 *s*	76.76 *s*	77.82 *s*	76.72 *s*
3	131.09/131.11 *d*	130.87/130.85 *d*	130.25/130.27 *d*	130.11 *d*
4	122.68 *d*	120.00 *d*	122.61 *d*	120.06 *d*
4a	121.46 *s*	120.29 *s*	118.90 *s*	116.06 *s*
5	110.21 *d*	116.74 *s*	109.76 *d*	117.78 *s*
6	148.47 *s*	146.55 *s*	146.84 *s*	145.24 *s*
7	116.97 *d*	116.67 *d*	123.45 *s*	122.31 *s*
8	126.42 *s*	123.42 *s*	125.24 *s*	122.89 *s*
8a	144.95 *s*	144.99 *s*	144.74 *s*	144.64 *s*
2-Me	25.75 *q*	25.34 *q*	25.66 *q*	25.25 *q*
5-Me		10.52 *q*		10.84 *q*
7-Me			12.08 *q*	12.41 *q*
8-Me	15.47 *q*	15.31 *q*	11.65 *q*	11.59 *q*
1′	41.00/40.96 *t*	40.61/40.65 *t*	40.95/40.91 *t*	40.55 *t*
2′	21.29/21.28 *t*	21.31/21.32 *t*	21.33/21.31 *t*	21.33 *t*
3′, 5′, 7′, 9′[a]	37.42, 37.34, 37.33, 37.27 *t*	37.43, 37.43, 37.33, 37.28 *t*	37.45, 37.43, 37.35, 37.26 *t*	37.48, 37.43, 37.34, 37.28 *t*
4′	32.67 *d*	32.69 *d*	32.68 *d*	32.70 *d*
6′	24.43 *t*	24.46 *t*	24.44 *t*	24.46 *t*
8′	32.79 *d*	32.79 *d*	32.79 *d*	32.80 *d*
10′	24.78 *t*	24.79 *t*	24.79 *d*	24.79 *t*
11′	39.37 *t*	39.37 *t*	39.37 *t*	39.37 *t*
12′	27.97 *d*	27.98 *d*	27.98 *d*	27.98 *d*
4′-Me	19.67/19.62 *q*	19.64/19.68 *q*	19.68/19.65 *q*	19.64 *q*
8′-Me	19.74 *q*	19.74 *q*	19.74 *q*	19.74 *q*
12′-Me	22.71 *q*	22.71 *q*	22.71 *q*	22.71 *q*
13′	22.62 *q*	22.62 *q*	22.62 *q*	22.62 *q*

[a] Interchangeable, possibly with overlapping diastereomeric splitting.

usable resonance pairs gave the same result confirmed this assumption. The corresponding diastereomeric shifts calculated from the ^{13}C shift values of Brownstein et al. (1989) for the natural (*R*,*R*,*R*) form by subtraction of the shift values for the C-2 diastereomer (*S*,*R*,*R*) [$\Delta = \delta_{RRR} - \delta_{SRR}$] were for C-1′ = −0.08, C-2′ = −0.03, and 4′-CH_3 = −0.04; the value for C-3 is not comparable with the chromene system of **1**. The absolute values agreed well, however all signs were reversed. This means that in the diastereomeric mixture of **1** the component with the unexpected (*S*,*R*,*R*) configuration predominated. In the ^1H NMR spectrum the diastereomeric mixture was indicated only by the resonance of the 4′-CH_3 dublett, which could not be analyzed quantitatively due to the overlapping 8′-CH_3 resonance (see Section 3). Nevertheless, it is rather interesting that the 4′-methyl group which is relatively far from C-2 showed a diastereomeric splitting in both the ^1H and ^{13}C NMR spectra. This was in agreement with Newman's "Rule of Six" which was discussed in detail in the paper of Brownstein et al. (1989) for the ^{13}C NMR data of diastereomeric α-tocopherols. The optical rotation of the diastereomeric mixture was $[\alpha]_D^{25} = +11°$ ($[\alpha]_{436}^{25} = +14°$). Compared to the tocopherol series the value for the dehydro derivative was significantly larger. Due to the simple benzene chromophor at some distance from the chiral centers, the optical rotations of the tocopherols are generally very small with $[\alpha]_{546}^{25}$ between +0.32 and +3.4 in ethanol (compare Baxter et al., 1943, and Stern et al., 1947 for the natural *R*,*R*,*R* series and Schudel et al., 1963 for the 2*S* diastereomer, *S*,*R*,*R*).

Compound **2**, isolated from *S. collinsae*, differed from **1** by a further methyl group leaving only one proton in the benzene ring. NOESY proved the substitution pattern of a 3,4-dehydro-β-tocopherol, all ^{13}C NMR assignments were again confirmed by HMBC and the structure was also compatible with EIMS and HREIMS (see Section 3). In analogy to **1**, ^1H NMR analysis showed that an impurity of ca. 10% of the corresponding β-tocopherol was present (4-H_2 δ = 2.60 ppm, 3-H_2 $\delta \approx 1.79$ ppm). Checking for diastereomeric twin peaks in the ^{13}C NMR spectrum revealed that in the case of **2** one diastereomer was highly dominant, the diastereomeric contamination of ⩽10% appeared only as rather small satellites. The optical rotation of product **2** was $[\alpha]_D^{25} = -21$ ($[\alpha]_{546}^{25} = -26°$), and after correction for 10% β-tocopherol a value of $[\alpha]_{546}^{25} = -29°$ could be calculated for pure **2**. The diastereomeric mixture of **2** with a ratio 90:10% was rather interesting in comparison to **1**. In the case of **2**, the diastereomeric shift differences in $CDCl_3$ were for C-3 = +0.02, C-1′ = −0.04, C-2′ = −0.01, and 4′-CH_3 = −0.04. This was consistently opposite to the values for **1** clearly indicating the high preponderance of the common (*R*,*R*,*R*) absolute configuration for **2**.

^1H NMR and MS analysis of **3**, isolated from *S. curtisii*, showed that it consisted of 99% 3,4-dehydro-γ-tocopherol with contaminations of ca. 1% γ-tocopherol (4-H_2 δ = 2.67 ppm, 3-H_2 1.74 ppm). The structure of the highly dominating 3,4-dehydro-γ-tocopherol (**3**) followed from comparison of the ^1H and ^{13}C NMR spectra with the spectra of **1** and **2** together with the mass spectral data (EIMS, HREIMS, see Section 3). The intensities of the ^{13}C NMR twin resonances for carbon atoms 3, 1′, 2′, and 4′-Me indicated a diastereomeric composition of ca. 60:40% for the (*S*,*R*,*R*) and the (*R*,*R*,*R*) form (Table 1). The optical rotations for product **3** were $[\alpha]_D^{25} = +11°$ and $[\alpha]_{546}^{25} = +13°$. The diastereomeric composition and the optical rotation values of dehydro-γ-tocopherol (**3**) matched exactly the results obtained for dehydro-δ-tocopherol (**1**). From *S. collinsae* **3** was isolated as a mixture with ca. 10% γ-tocopherol and ca. 10% of the isomeric 3,4-dehydro-β-tocopherol (**2**).

In the ^1H NMR spectrum of compound **4** from *S. collinsae* three aromatic methyl resonances were observed for the main component of 80% purity. A comparison of all ^1H and ^{13}C NMR as well as MS data with those of Lei and Atkinson (2000) proved that **4** was 3,4-dehydro-α-tocopherol accompanied with 20% of the corresponding α-tocopherol (4-H_2 δ = 2.60 ppm, 3-H_2 1.78 ppm). In contrast to the dehydrotocopherols **1**–**3** compound **4** proved to be diastereomerically pure, since no diastereomeric satellites appeared in the ^{13}C NMR spectrum. Comparison of the ^{13}C NMR shift values for C-2′ (21.33 ppm) and 4′-Me (19.64) with the corresponding data of the (*R*,*R*,*R*) forms of **2** and **3** (Table 1) indicated that in the case of dehydro-α-tocopherol (**4**) only the usual (*R*,*R*,*R*) absolute configuration was present. The optical rotations were $[\alpha]_D^{25} = -18°$, ($[\alpha]_{546}^{25} = -22°$), and after correction for 20% α-tocopherol a value of $[\alpha]_{546}^{25} = -28°$ may be calculated for pure compound **4**.

Several properties were influenced by the steric interaction of the methyl group at position 5 with the neighbouring olefinic/aromatic proton 4-H. This interaction seems to lead to different conformations of the second (pyrane) ring of the chromene systems of **1**–**4**. The change of conformation in compounds **2** and **4** with a 5-CH_3 group, compared to compounds **1** and **3** with a less space-consuming 5-H was clearly reflected in the UV spectra of compounds **2** and **4** compared to **1** and **3**, showing small, but significant differences (see Fig. 1 and Section 3). The same was true for the NMR resonances, e.g., for the ^1H chemical shifts of 4-H: 6.53 and 6.52 ppm for **2** and **4**, but 6.24 and 6.22 for **1** and **3**, indicating a slightly different position relative to the benzene ring. A further striking difference between **2** and **4** compared to **1** and **3** was the chiroptical behaviour. The $[\alpha]_{546}^{25}$ values (corrected) were −29° and −28° for **2** and **4**, and +14° and +13° for **1** and **3**, respectively. However, it should be noted that (*R*,*R*,*R*)-**2** and -**4** were

only slightly contaminated with the (*S*,*R*,*R*) diastereomer or even diastereomerically pure, and the isolated products **1** and **3** were present in mixtures (*S*,*R*,*R*):(*R*,*R*,*R*) of ca. 60:40% with a preponderance of the (*S*,*R*,*R*) diastereomer.

2.2. Accumulation trends and distribution

Due to their extended conjugated system chromenols could easily be distinguished from the corresponding chromanol derivatives in the more lipophilic column fractions by their characteristic UV spectra (Fig. 1). Based on our current comparative HPLC–UV-analyses within the family Stemonaceae (Pacher et al., 2002; Brem et al., 2002; Kaltenegger et al., 2003) it became evident that the formation of dehydrotocopherols is typical for many *Stemona* species, where it predominates in the tuberous roots mostly accompanied by smaller amounts of corresponding saturated tocopherols. However, because of very similar UV spectra and retention times of some derivatives their distribution in 20 geographical provenances from about 10 different *Stemona* species could not be sufficiently determined in the HPLC profiles on its own. An overview about the distribution and various accumulation trends of all derivatives could only be achieved by the combination of HPLC with comparative GC–MS and TLC analyses. Since all compounds isolated by preparative MPLC and TLC represented mixtures of chromenols with different amounts of corresponding chromanols, their quantitative composition was ascertained by calculation using NMR data (compare footnotes in Table 3).

Based on NMR data of the isolated compounds and comparative GC–MS analyses of crude extracts and lipophilic column fractions various accumulation trends were observed towards distinct methylations of the aromatic ring representing chemical markers for the different species. As shown in Table 2, they were largely in line with the presently accepted species delimitations (Duyfjes, 1993; Tsi and Duyfjes, 2000). However, all chromenol derivatives detected in the present investigation were shown to be overlapped mostly by smaller amounts of corresponding chromanols. By contrast, the two different provenances of *Stemona kerrii* Craib clearly differed due to the preponderance of the chromanol derivatives δ- and especially γ-tocopherol, accompanied only by traces of the corresponding dehydrotocopherols **1** and **3**. Similarly, *Stemona cochinchinensis* Gagnep. was also characterized by the dominating chromanol γ-tocopherol together with only smaller amounts of the corresponding chromenol derivative **3**. *Stemona* cf. *pierrei* Gagnep. appeared to be taxonomically more separated (Kostecki et al., 2004), because no tocopherols could be detected at all (Table

Table 2
Accumulation trends of dehydrotocopherols (chromenols **1**–**4**) in the underground parts of various *Stemona* species and provenances[a]

Stemona species	Provenances	Chromenol derivatives			
		1	**2**	**3**	**4**
S. curtisii	(HG 899) S-Thailand, Pacho			○	+
	(HG 917) S-Thailand, Narathiwat	+		○	+
	(HG 911) S-Thailand, Krabi	+		•	+
	(HG 865) S-Thailand, Satun	+		○	+
	(HG 920) S-Thailand, Chumphon			○	+
S. burkillii	(HG 887) N-Thailand, Chiang Mai				+
S. tuberosa	(HG 851) SO-Thailand, Rayong	•			
	(HG 890) N-Thailand, Chiang Mai	•			
	(HG 879) Vietnam, Hanoi	•			
	(HG 918) Indonesia, Bali	•			
	(HG 919) Bangkok, local market	•			
S. cochinchinensis	(HG 884) NE-Thailand, Nong Khai			○	
S. collinsae	(HG 840) SE-Thailand, Chon Buri	•	○	+	+
S. undet. species	(HG 915) NE-Thailand, Udon Thani	+	+		○
	(HG 896) E-Thailand, N. Ratchasima			+	○
	(HG 893) N-Thailand, Sukhothai				○
	(HG 913) W-Thailand, Kanchanaburi	+		+	○
S. kerrii	(HG 889) N-Thailand, Chiang Mai (HG 892) NW-Thailand, Tak	Mainly chromanol derivatives accumulated			
S. cf. *pierrei*	(HG 910) E-Thailand, Sri Saket	No tocopherols detected			

[a] The symbols represent an estimate of the relative abundance on the basis of preparative analyses (*S. curtisii*, *S. tuberosa*, *S. collinsae*) and/or total ion concentration (TIC) and single ion traces from the GC–MS obtained from crude extracts or lipophilic column fractions (10–25% EtOAc in hexane). •, major quantity (>10 mg/g extract); ○, minor quantity (≥2 mg/g extract); +, small quantity (<2 mg/g extract).

2). A clear accumulation trend towards dehydro-γ-tocopherol (**3**) was found in all five provenances of *S. curtisii* Hook.f. collected in S-Thailand, accompanied by small amounts of dehydro-α-tocopherol (**4**) and traces of dehydro-δ-tocopherol (**1**). However, the total amounts significantly differed between the provenances. On the other hand, the five representatives of the *S. tuberosa* group, most likely comprising four different subspecies or varieties on the basis of floral and chemical characters, were uniformly characterized by the sole occurrence of great amounts of dehydro-δ-tocopherol (**1**) containing only 1% of δ-tocopherol. Compound **1** was also dominating in *S. collinsae*, but was accompanied here by minor quantities of the rare dehydro-β-tocopherol (**2**) and small amounts of **3** and **4**. In *Stemona burkillii* D.Prain from the mountains of N-Thailand and the group of unidentified species HG 915, HG 896, HG 893, and HG 913 a weak but consistent accumulation trend towards dehydro-α-tocopherol (**4**) could be detected. Morphological and chemical characters of the latter group suggest the presence of at least three different species from which the characteristic alkaloid profile of HG 915 has already been published recently (Kaltenegger et al., 2003). HG 896 could be clearly distinguished as a separate species by different floral characters, whereas HG 893 and HG 913 probably belong to one species only.

Since different plant tissues were known to vary enormously in their total tocopherol content (Grusak and DellaPenna, 1999), more detailed investigations were carried out in *Stemona* species to detect the place of accumulation. In *S. tuberosa* higher amounts of tocopherols were only found in the underground parts where they were exclusively accumulated in the outer layers of the root bark. In *S. collinsae* they were shown to be restricted to the older roots characterised by a brownish outer root bark, whereas in the young white tubers no tocopherols could be detected.

With respect to their biosynthetic origin tocopherols can be regarded as products of two different pathways involving homogentisinic acid and phytyl diphosphate (Stocker et al., 1993, 1994; Lichtenthaler, 1999; Grusak and DellaPenna, 1999; Munné-Bosch and Alegre, 2002). Whereas the aromatic methyl group at C-8 of δ-tocopherol can be directly derived from homogentisinic acid by decarboxylation, the additional methylations leading to γ-, β-, and α-tocopherols were inserted by specific methyltransferases. Based on the properties of the two methyltransferases involved in tocopherol synthesis the different patterns, shown in Table 2, apparently reflect different methyltransferase activities especially of the one that converts the 2-methyl-6-phytyl-1,4-benzoquinone to the respective dimethyl derivative. This enzymatic activity effects the accumulation of γ types whereas a lack results in the formation of δ types. By contrast, the conversion of δ to β or γ to α types requires the activity of the tocopherol methyltransferase which has low but significant activity in certain *Stemona* species and appears to display a selectivity for the (*R*,*R*,*R*) forms. Regarding the co-occurrence of all tocopherol types with the corresponding dehydro derivatives, the formation of the characteristic double bond between C-3 and C-4 apparently respresents a later biosynthetic step.

2.3. Antioxidant activity

In a preliminary screening thin-layer chromatography experiments were performed to test antiradical activity of dehydrotocopherols **1**–**4**. After staining the TLC-plates with 0.4 mM DPPH· in methanol, all samples showed clear inhibition zones at quantities of 10 μg comparable with that of α-tocopherol (**5**) (data not shown). To get more detailed information about the different antiradical capacities, kinetic assays were performed in microplates to record spectrophotometrically the varying decrease of absorbance of DPPH· at 550 nm, and α-tocopherol (**5**) served as positive control. The results listed in Table 3 confirmed the radical scavenger activities of dehydrotocopherols **1**–**4**, already indicated by TLC experiments, and showed only slight differences among each other. Comparing the values of the tocopherol **5** with the corresponding dehydro derivative **4** it can be concluded that the insertion of a double bond between the C-3 and C-4 position has no significant influence on the antioxidative capacity. In terms of reaction kinetics the position of the methyl groups in the aromatic ring plays a crucial role in the hydrogen donating ability. Yen and Duh (1994) postulated that the more rapidly the absorbance decreased, the more potent were the antioxidant activity of the sample. In case

Table 3
Scavenging activities of dehydrotocopherols **1**–**4**, compared with α-tocopherol (**5**) on DPPH· radicals[a]

Compounds	EC_{50} (95% FL[b]) (μM)	EC_{50} (95% FL[b]) (ppm)
Dehydro-δ-tocopherol (**1**)[c]	25 (21.5–29.0)	10 (8.6–11.6)
Dehydro-β-tocopherol (**2**)[d]	25 (21.2–28.2)	10 (8.8–11.7)
Dehydro-γ-tocopherol (**3**)[e]	21 (14.5–29.2)	9 (6.1–12.1)
Dehydro-α-tocopherol (**4**)[f]	22 (15.9–30.8)	9 (6.8–13.2)
α-Tocopherol (**5**)	20 (17.6–22.8)	9 (7.6–9.8)

[a] An ethanolic DPPH· solution with a final concentration of 100 μM was mixed with different concentrations of tocopherols and the absorbance change at 550 nm was measured in a time course with an ELISA-reader. Inhibition of coloration was expressed as percentage, and EC_{50} values were obtained from inhibition curves after 90 min reaction time.
[b] Fiducial limits.
[c] Contaminated with 4% δ-tocopherol.
[d] 10% β-tocopherol.
[e] 1% γ-tocopherol.
[f] 20% α-tocopherol.

of the tocopherols, compounds **4** and **5**, with three aromatic methyl groups, showed a very rapid kinetic behaviour, whereas **1**–**3** exhibited only a slow decrease in absorbance. Even at a measurement immediately after preparing the plate, **4** and **5** already showed EC_{50} values of 15 and 13 ppm, and after 5 min the plateau was nearly reached with 10 and 9 ppm, respectively. In contrast, immediate measurements of **1**–**3** resulted in significantly higher EC_{50} values of 50, 51 and 36 ppm and even after 5 min the scavenging activity was still less pronounced with 18, 16 and 19 ppm. All 5 tocopherols reached a plateau after 90 min. In conclusion, the optimal configuration for a highly efficient activity in vitro was, in accordance to the corresponding chromanols (Kamal-Eldin and Appelqvist, 1996), the concomitant methylation of the C-5 and C-7 position in dehydro-α-tocopherol (**4**).

3. Experimental

3.1. General

NMR: Bruker DRX400 WB ($CDCl_3$ ^1H δ 7.26; ^{13}C δ 77.0; acetone-d_6 ^1H δ 2.05, ^{13}C δ 29.84). MS: Finnigan MAT 900 S. IR: Perkin–Elmer 16PC FT-IR. HPLC: Hewlett–Packard 1090 Series II, UV diode array detection at 230 nm, column 150 (4.6 mm, Phenomenex Synergi Polar-RP80A), 4 μm, mobile phase acetonitrile (gradient 20–60%) in aqueous buffer (0.015 M *o*-phosphoric acid, 0.0015 M tetrabutylammonium hydroxide, pH 3), flow rate 1 ml/min. GC–MS: Perkin–Elmer GC Autosystem XL linked to a Turbomass quadrupole MS.

3.2. Plant material

Twenty different provenances representing *S. curtisii* (HG 865, HG 899, HG 911, HG 917, HG 920), *S. burkillii* (HG 887), *S. tuberosa* (HG 851, HG 879, HG 890, HG 918, HG 919), *S. cochinchinensis* (HG 884), *S. collinsae* (HG 840), *S. kerrii* (HG 889; HG 892), and *S.* cf. *pierrei* (HG 910) as well as the unidentified species HG 893, HG 896, HG 913, HG 915 were collected from Indonesia, Vietnam, and various localities in Thailand (Table 2). Voucher specimens were deposited at the Herbarium of the Institute of Botany, University of Vienna (WU).

3.3. Extraction and isolation

Underground parts (including roots and rhizomes) from *Stemona collinsae* (HG 840), *S. tuberosa* (HG 851), and *S. curtisii* (HG 917) were dried for 3–4 weeks and ground separately. The homogenates were extracted with MeOH at room temp. for 5 days, filtered and conc. The aqueous residues were extracted with $CHCl_3$, and the concentrated $CHCl_3$ fractions were used as lipophilic crude extracts for comparative HPLC and GC–MS analyses. From *S. collinsae* 4.7 g extract obtained from ca. 600 g dried underground parts were roughly separated by CC (Merck Si gel 60, 35–70 mesh). The lipophilic fractions eluted with 10–25% EtOAc in hexane (800 mg) were further separated by preparative MPLC (400 x 40 mm column, Merck LiChroprep silica 60, 25–40 μm, UV detection, 254 nm) with 5–10% EtOAc in hexane to give four fractions corresponding to compounds **1**–**4** with high impurity (I–IV): fr I = 5 mg, fr II = 20 mg, fr III = 7 mg, fr IV = 60 mg. Final purification of each by prep. TLC (toluene-Et_2O = 97:3) afforded 1.5 mg of **4**, 5 mg of **2**, 2 mg of **3**, and 33 mg of **1**. From 180 g underground parts of *S. tuberosa* corresponding isolation procedures led to 14 mg **1**, and from 160 g underground parts of *S. curtisii* to 0.5 mg **4** and 5 mg **3**.

3.4. 3,4-Dehydro-δ-tocopherol. 6-Hydroxy-2,8-dimethyl-2-(4,8,12-trimethyldecyl)-2H-chromen (*1*)

Diastereomeric mixture (*S*,*R*,*R*):(*R*,*R*,*R*) ca. 60:40% (plus a contamination of ca. 4% δ-tocopherol). $[\alpha]_D^{25} = +11°$, $[\alpha]_{546}^{25} = +14°$, $[\alpha]_{436}^{25} = +27°$ (EtOH, $c = 1.0$). UV λ^{MeOH} 334, 274 sh, 264, 232 nm. IR ν^{CCl4} cm^{-1} 3616 m, 2954 s, 2925 s, 2867 m, 1464 s, 1378 m, 1366 w, 1316 m, 1239 m, 1182 m, 1147 w, 1130 w, 996 w, 927 w, 860 w. ^1H NMR ($CDCl_3$) $\delta = 6.47$ (*d*, 1H, $J = 2.9$ Hz, 7-H), 6.32 (*d*, 1H, $J = 2.9$ Hz, 5-H), 6.24 (*d*, 1H, $J = 9.7$ Hz, 4-H), 5.59 (*d*, 1H, $J = 9.7$ Hz, 3-H), 4.32 (br.*s*, 1H, OH), 2.13 (*s*, 3H, 8-CH_3), 1.62 (*m*, 2H, 1′-H_2), 1.53 (*nonett*, 1H, $J = 6.6$ Hz, 12′-H), 1.35 (*s*, 3H, 2-CH_3), 1.0–1.4 (*m*, 18H, 2′-11′-H_2 or -H), 0.87 (*d*, 6H, $J = 6.6$ Hz, 12′-CH_3 and 13′-H_3), 0.839 (*d*, 3H, $J = 6.6$ Hz, 8′-CH_3), 0.839/0.833 (*d*, total 3H, $J = 6.6$ Hz, 4′-CH_3). ^{13}C NMR ($CDCl_3$), see Table 1. NOESY: chain of contacts 8-CH_3 ↔ 7-H ↔ 6-OH ↔ 5-H ↔ 4-H ↔ 3-H. HMBC: C-2 → 3-H, 4-H, 2-CH_3; C-3 → 2-CH_3; C-4a → 3-H, 4-H; C-5 → 4-H, 7-H, OH; C-6 → OH; C-7 → 5-H, OH, 8-CH_3; C-8 → 8-CH_3; C-8a → 4-H, 8-CH_3; 8-CH_3 → 7-H. ^1H NMR (acetone-d_6) $\delta = 7.61$ (br.*s*, 1H, OH), 6.49 (*d*, 1H, $J = 2.8$ Hz, 7-H), 6.36 (*d*, 1H, $J = 2.8$ Hz, 5-H), 6.30 (*d*, 1H, $J = 9.8$ Hz, 4-H), 5.66 (*d*, 1H, $J = 9.8$ Hz, 3-H), 2.08 (*s*, 3H, 8-CH_3), 1.64 (*m*, 2H, 1′-H_2), 1.54 (*nonett*, 1H, $J = 6.6$ Hz, 12′-H), 1.33 (*s*, 3H, 2-CH_3), 1.1-1.5 (*m*, 18H, 2′-11′-H_2 or -H), 0.87 (*d*, 6H, $J = 6.6$ Hz, 12′- and 13′-CH_3), 0.858 (*d*, 3H, $J = 6.6$ Hz, 8′-CH_3), 0.858/0.852 (*d*, total 3H, $J = 6.6$ Hz, 4′-CH_3). ^{13}C NMR (acetone-d_6, diastereomers ca. 60/40%) $\delta = 151.40$ (*s*, C-6), 144.82 (*s*, C-8a), 131.58/131.61 (*d*, C-3), 126.36 (*s*, C-8), 123.84 (*d*, C-4), 122.30 (*s*, C-4a), 117.81 (*d*, C-7), 111.66 (*s*, C-5), 78.46/78.45 (*s*, C-2), 41.68/41.63 (*t*, C-1′), 40.11 (*t*, C-11′); 38.14, 38.13, 38.09, 38.07, 38.03, and 38.00 (C-3′, -5′, 7′, and 9′, with overlapping diastereomeric

splitting); 33.51 (*d*, C-8′), 33.41 (*d*, C-4′), 28.68 (*d*, C-12′), 26.12/26.09 (*q*, 2-CH_3), 25.51 (*t*, C-10′), 25.10/25.11 (*t*, C-6′), 23.00 (*q*, 12′-CH_3), 22.91 (*q*, C-13′), 22.06/22.02 (*t*, C-2′), 20.10 (*q*, 8′-CH_3), 20.08/20.04 (*q*, 4′-CH_3), 15.65 (*q*, 8-CH_3). EIMS (70 eV) *m/z* 400 (7%, M^+), 385 (5, $M^+ - CH_3$), 175 (100, M^+ – side chain ≡ $C_{11}H_{11}O_2$), 57 (9). HR-EIMS *m/z* 400.3346 (calcd for $C_{27}H_{44}O_2$ 400.3341).

3.5. 3,4-Dehydro-β-tocopherol. 6-Hydroxy-2,5,8-trimethyl-2-(4,8,12-trimethyldecyl)-2H-chromen (2)

Diastereomeric mixture (*R*,*R*,*R*):(*S*,*R*,*R*) ca. 90:10 (plus ca. 10% β-tocopherol). $[\alpha]_D^{25} = -21°$, $[\alpha]_{546}^{25} = -26°$, $[\alpha]_{436}^{25} = -59°$, and $[\alpha]_{546}^{25}$ (corr. for pure diastereomeric mixture **2**) = −29°, (Et OH, *c* = 0.2). UV λ^{MeOH} 338, 280, 270, 232 nm. IR ν^{CCl4} cm^{-1} 3619 m, 2955 s, 2928 s, 2870 m, 1463 s, 1418 w, 1377 m, 1286 m, 1240 m, 1139 w, 1122 w, 1073 w, 1057 m, 983 w, 922 w, 872 w. ^{1}H NMR ($CDCl_3$) δ = 6.46 (*s*, 1H, 7-H), 6.53 (*d*, 1H, *J* = 10.1 Hz, 4-H), 5.64 (*d*, 1H, *J* = 10.1 Hz, 3-H), 4.20 (br.*s*, 1H, OH), 2.16 (*s*, 3H, 5-CH_3), 2.11 (*s*, 3H, 8-CH_3), 1.61 (*m*, 2H, 1′-H_2), 1.52 (*nonett*, 1H, *J* = 6.6 Hz, 12′-H), 1.33 (*s*, 3H, 2-CH_3), 1.0-1.45 (*m*, 18H, 2′-11′-H_2 or -H), 0.87 (*d*, 6H, *J* = 6.6 Hz, 12′-CH_3 and 13′-H_3), 0.84 (2 × *d*, 6H, *J* = 6.6 Hz, 4′- and 8′-CH_3). ^{13}C NMR ($CDCl_3$), see Table 1. NOESY: 8-CH_3 ↔ 7-H ↔ 6-OH; 5-CH_3 ↔ 4-H ↔ 3-H. HMBC: C-2 → 3-H, 4-H, 2-CH_3; C-3 → 2-CH_3; C-4a → 3-H, 4-H; C-5 → 4-H, 7-H, OH, 5-CH_3; C-6 → OH; C-7 → OH, 8-CH_3; C-8 → 8-CH_3; C-8a → 4-H, 8-CH_3; 8-CH_3 → 7-H. EIMS (70 eV) *m/z* 414 (7%, M^+), 399 (4, $M^+ - CH_3$) 189 (100, M^+ – side chain ≡ $C_{12}H_{13}O_2$), 149 (23), 57 (10). HR-EIMS *m/z* 414.3501 (calcd for $C_{28}H_{46}O_2$ 414.3498).

3.6. 3,4-Dehydro-γ-tocopherol. 6-Hydroxy-2,7,8-trimethyl-2-(4,8,12-trimethyldecyl)-2H-chromen (3)

Diastereomeric mixture (*S*,*R*,*R*):(*R*,*R*,*R*) ca. 60:40% (plus a contamination of 1% γ-tocopherol). $[\alpha]_D^{25} = +11°$, $[\alpha]_{546}^{25} = +13°$, $[\alpha]_{436}^{25} = +25°$ (EtOH, *c* = 0.2). UV λ^{MeOH} 332, 278 sh, 268, 232 nm. IR ν^{CCl4} cm^{-1} 3619 m, 2953 s, 2927 s, 2868 m, 1463 s, 1428 m, 1377 m, 1314 w, 1238 m, 1220 m, 1158 w, 1103 w, 1080 s, 927 w, 876 w. ^{1}H NMR ($CDCl_3$) δ = 6.31 (*s*, 1H, 5-H), 6.22 (*d*, 1H, *J* = 9.8 Hz, 4-H), 5.54 (*d*, 1H, *J* = 9.8 Hz, 3-H), 4.22 (br.*s*, 1H, OH), 2.14 (*s*, 3H, 7-CH_3), 2.12 (*s*, 3H, 8-CH_3), 1.61 (*m*, 2H, 1′-H_2), 1.52 (*nonett*, 1H, *J* = 6.6 Hz, 12′-H), 1.34 (*s*, 3H, 2-CH_3), 1.0-1.4 (*m*, 18H, 2′-11′-H_2 or -H), 0.86 (*d*, 6H, *J* = 6.6 Hz, 12′-CH_3 and 13′-H_3), 0.83 (2 × *d*, 6H, *J* = 6.6 Hz, 4′- and 8′-CH_3). ^{13}C NMR ($CDCl_3$), see Table 1. EIMS (70 eV) *m/z* 414 (7%, M^+), 399 (4, $M^+ - CH_3$) 189 (100, M^+ – side chain ≡ $C_{12}H_{13}O_2$), 151 (7), 57 (12). HREIMS *m/z* 414.3500 (calcd for $C_{28}H_{46}O_2$ 414.3498).

3.7. 3,4-Dehydro-α-tocopherol. 6-Hydroxy-2,5,7,8-tetramethyl-2-(4,8,12-trimethyldecyl)-2H-chromen (4)

Diastereomerically pure (*R*,*R*,*R*) product, with a contamination of ca. 20% α-tocopherol. $[\alpha]_D^{25} = -18°$, $[\alpha]_{546}^{25} = -22°$, $[\alpha]_{436}^{25} = -56°$, and $[\alpha]_{546}^{25}$ (corr. for pure compound **4**) = −28°, (MeOH, *c* = 0.1). UV λ^{MeOH} 332, 282, 272, 232 nm. IR ν^{CCl4} cm^{-1} 3626 m, 2952 s, 2925 s, 2867 m, 1461 s, 1419 w, 1377 m, 1339 w, 1266 m, 1212 m, 1169 w, 1113 w, 1086 m, 1060 w, 924 w, 866 w. ^{1}H NMR ($CDCl_3$) δ = 6.52 (*d*, 1H, *J* = 10.1 Hz, 4-H), 5.61 (*d*, 1H, *J* = 10.1 Hz, 3-H), 4.18 (br.*s*, 1H, OH), 2.18 (*s*, 3H, 5-CH_3), 2.15 (*s*, 3H, 7-CH_3), 2.11 (*s*, 3H, 8-CH_3), 1.60 (*m*, 2H, 1′-H_2), 1.52 (*nonett*, 1H, *J* = 6.6 Hz, 12′-H), 1.33 (*s*, 3H, 2-CH_3), 1.0-1.4 (*m*, 18H, 2′-11′-H_2 or -H), 0.87 (*d*, 6H, *J* = 6.6 Hz, 12′-CH_3 and 13′-H_3), 0.84 (2*d*, 6H, *J* = 6.6 Hz, 4′- and 8′-CH_3). ^{13}C NMR ($CDCl_3$), see Table 1. EIMS (70 eV) *m/z* 428 (10%, M^+), 413 (5, $M^+ - CH_3$), 203 (100, M^+ – side chain ≡ $C_{13}H_{15}O_2$), 165 (10), 57 (6). HREIMS *m/z* 428.3651 (calcd for $C_{29}H_{48}O_2$ 428.3654).

3.8. Comparative GC–MS-analyses

Crude extracts or lipophilic column fractions were silylated with BSTFA–TMCS (99:1) in pyridine at ambient temperature (concentration range 1–5 μg/ml). Injection was performed in the splitless mode (0.5 μl, injector temperature 250 °C). The used columns were a PE-5ms (20 m, 0.18 mm diameter, 0.18 μm film) and PE-1704 (15 m, 0.25 mm diameter, 0.25 μm film). The carrier helium was kept at a constant flow rate of 1 ml/min. The ion source was thermostated to 180 °C and the transfer line to 250 °C. EI spectra were recorded from 30 to 600 amu at an electron energy of 70 eV and a filament emission of 200 μA. The temperature gradient of the oven started at 75 °C and rose with 5 °C/min to 300 °C. Tocopherols and dehydrotocopherols were identified by comparing single ion traces (*m/z* = 502 for α-tocopherol, *m/z* = 488 for β-tocopherol and γ-tocopherol, *m/z* = 474 for δ-tocopherol, *m/z* = 275 for dehydro-α-tocopherol, *m/z* = 261 for dehydro-β-tocopherol and dehydro-γ-tocopherol, and *m/z* = 247 for dehydro-δ-tocopherol). The symbols in Table 2 represent an estimate of the relative abundance on the basis of total ion concentration (TIC) and single ion traces from the GC–MS.

3.9. Antioxidant tests with TLC

For rapid visualization of antiradical acitivity 10 μg of pure compounds were applied on 0.25 mm silica gel 60 F254 TLC plates (Macherey-Nagel, Düren, Germany). A mixture of hexan/diethylether (85:15) was used as mobile phase. The plate was stained with 0.4 mM DPPH· in methanol. Active samples appeared as light spots against a purple background (results not shown).

3.10. Microplate assay for radical scavenging activity

The experimental procedure was adapted from Brand-Williams et al. (1995) and Matsukawa et al. (1997). In this assay, the stable free radical 2,2-diphenyl-1-picryl hydrazyl (DPPH·), which has a strong absorption at 550 nm, reacts with antioxidants and produces colourless 2,2-diphenyl-1-picryl hydrazine independently of enzymatic activities. Dilution series of test compounds, dissolved in EtOH, were performed in sterile disposable microtiter plates (96 U-shaped wells, Greiner Labortechnik, Kremsmünster, Austria), using freshly prepared 100 μM DPPH·/ethanol solutions. α-Tocopherol (Sigma–Aldrich, Austria) served as positive control. Pure compounds were tested in two-fold serial dilutions ranging from 3 to 200 ppm with a final volume of 200 μl for all of the assays. For each pure compound, a total of six tests were attempted, including three independent sample preparations. Results were determined after 0, 5, 10, 20, 30, 60, 90, 120 and 240 min of reaction time in order to analyse antiradical activities at the steady state. However, due to the high volatility of organic solvents, all tests were stopped after 4 h. The drug-related disappearance of the free radical DPPH· was measured spectrophotometrically at 550 nm with an ELISA reader (Reader 400 ATC, SLT – Labinstruments Ges.m.b.H., Grödig, Austria), online interfaced to an IBM Thinkpad. The percentage inhibition was defined by the absorbance of the blank samples (200 μl 100 μM DPPH·/EtOH) to the DPPH·/EtOH solutions containing different concentrations of samples. Since the kinetic assays followed a log-normal pattern, the log concentration–response probit method was used for data analysis as described by Norusis (1999). The results determined after 90 min of reaction time were summarized in Table 3, including effective concentrations as well as their 95% confidence intervals.

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References

Asgill, J.O., Crombie, L., Whiting, D.A., 1978. Chromenylation of 2-naphthol and alkylhydroquinones: short syntheses of (2RS,4′R,8′R)-α-tocopherol (vitamin E) and (2RS,4′R,8′R)-β-tocopherol. Journal of the Chemical Society, Chemical Communications, 59–60.

Baxter, J.G., Robeson, C.D., Taylor, J.D., Lehman, R.W., 1943. Natural α-, β- and γ-tocopherols and certain esters of physiological interest. Journal of the American Chemical Society 65, 918–924.

Brand-Williams, W., Cuvelier, M.E., Berset, C., 1995. Use of a free radical method to evaluate antioxidant activity. Lebensmittel-Wissenschaft und -Technologie 28, 25–30.

Brem, B., Seger, C., Pacher, T., Hofer, O., Vajrodaya, S., Greger, H., 2002. Feeding deterrence and contact toxicity of *Stemona* alkaloids – a source of potent natural insecticides. Journal of Agricultural and Food Chemistry 50, 6383–6388.

Brownstein, S., Burton, G.W., Hughes, L., Ingold, K.U., 1989. Chiral effects on the ^{13}C resonances of α-tocopherol and related compounds. A novel illustration of Newman's Rule of Six. Journal of Organic Chemistry 54, 560–569.

Duyfjes, B.E.E., 1993. Stemonaceae. Flora Malesiana Ser. I 11, 399–409.

Eitenmiller, R.R., 1997. Vitamin E content of fats and oils – nutritional implications. Food Technology 51, 78–81.

Espín, L.C., Soler-Rivas, C., Wichers, H.J., 2000. Characterization of the total free radical scavenger capacity of vegetable oils and oil fractions using 2,2-diphenyl-1-picrylhydrazyl radical. Journal of Agricultural and Food Chemistry 48, 648–656.

Grusak, M.A., DellaPenna, D., 1999. Improving the nutrient composition of plants to enhance human nutrition and health. Annual Review of Plant Physiology and Plant Molecular Biology 50, 133–161.

Kaltenegger, E., Brem, B., Mereiter, K., Kalchhauser, H., Kählig, H., Hofer, O., Vajrodaya, S., Greger, H., 2003. Insecticidal pyrido[1,2-a]azepine alkaloids and related derivatives from *Stemona* species. Phytochemistry 63, 803–816.

Kamal-Eldin, A., Appelqvist, L., 1996. The chemistry and antioxidant properties of tocopherols and tocotrienols. Lipids 31, 671–701.

Kostecki, K., Engelmeier, D., Pacher, T., Hofer, O., Vajrodaya, S., Greger, H., 2004. Dihydrophenanthrenes and other antifungal stilbenoids from *Stemona* cf. *pierrei*. Phytochemistry 65, 99–106.

Kurechi, T., Kikugawa, K., Kato, T., 1980. Studies on the antioxidants. XIII. Hydrogen donation capability of antioxidants to 2,2-diphenyl-1-picrylhydrazyl. Chemical Pharmaceutical Bulletin 28, 2089–2093.

Lei, H., Atkinson, J., 2000. Synthesis of phytyl- and chroman-derivatized photoaffinity labels based on α-tocopherol. Journal of Organic Chemistry 65, 2560–2567.

Lichtenthaler, H.K., 1968. Verbreitung und relative Konzentration der lipophilen Plastidenchinone in grünen Pflanzen. Planta 81, 140–152.

Lichtenthaler, H.K., 1999. The 1-deoxy-D-xylulose-5-phosphate pathway of isoprenoid biosynthesis in plants. Annual Review of Plant Physiology and Plant Molecular Biology 50, 47–65.

Matsukawa, R., Dubinsky, Z., Kishimoto, E., Masaki, K., Masuda, Y., Takeuchi, T., Chihara, M., Yamamoto, Y., Niki, E., Karube, I., 1997. A comparison of screening methods for antioxidant activity in seaweeds. Journal of Applied Phycology 9, 29–35.

McHale, D., Green, J., 1965. Chrom-3-en-6-ols. The action of pyridine on alk-2-enyl-benzoquinones. Journal of the Chemical Society, Chemical Communications, 5060–5064.

Munné-Bosch, S., Alegre, L., 2002. The function of tocopherols and tocotrienols in plants. Critical Reviews in Plant Sciences 21, 31–57.

Niki, E., Noguchi, N., 2004. Dynamics of antioxidant action of vitamin E. Accounts of Chemical Research 37, 45–51.

Norusis, M.J., 1999. SPSS Advanced Models 9.0. SPSS Inc., Chicago, IL.

Omura, K., 1989. Iodine oxidation of α-tocopherol and its model compound in alkaline methanol: unexpected isomerization of the product quinone monoketals. Journal of Organic Chemistry 54, 1987–1990.

Pacher, T., Seger, C., Engelmeier, D., Vajrodaya, S., Hofer, O., Greger, H., 2002. Antifungal stilbenoids from *Stemona collinsae*. Journal of Natural Products 65, 820–827.

Schudel, P., Mayer, H., Metzger, J., Rüegg, R., Isler, O., 1963. Die Umkehrung der Konfiguration am Kohlenstoffatom 2 von natürli-

chem (2*R*,4′*R*, 8′*R*)-α-tocopherol. Helvetica Chimica Acta 46, 333–343.

Stern, M.H., Robeson, C.D., Weisler, L., Baxter, J.G., 1947. δ-Tocopherol. I. Isolation from soybean oil and properties. Journal of the American Chemical Society 69, 869–874.

Stocker, A., Rüttimann, A., Woggon, W.D., 1993. Identification of the tocopherol cyclase in the blue-green algae *Anabaena variabilis* Kützing (Cyanobacteria). Helvetica Chimica Acta 76, 1729–1738.

Stocker, A., Netscher, T., Rüttimann, A., Müller, R.K., Schneider, H., Todaro, L.J., Derungs, G., Woggon, W.D., 1994. The reaction mechanism of chromanol-ring formation catalyzed by tocopherol cyclase from *Anabaena variabilis* Kützing (Cyanobacteria). Helvetica Chimica Acta 77, 1721–1737.

Tsi, Z.H., Duyfjes, B.E.E., 2000. Stemonaceae. In: Wu, Z.Y., Raven, P. (Eds.), Flora of China, vol. 24. Science Press, Beijing, pp. 70–72.

Yen, G.C., Duh, P.D., 1994. Scavenging effect of methanolic extracts of peanut hulls on free-radical and active-oxygen species. Journal of Agricultural and Food Chemistry 42, 629–632.