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Fatty acids and tocochromanols in seeds of Orobanche

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Abstract

The evaluation of tocochromanols (tocopherols and tocotrienols) in 49 accessions from 21 *Orobanche* species revealed three well separated groups. The first one, characterized by high γ-tocotrienol content, included all the accessions of sect. *Orobanche*. The second one, exhibiting high γ-tocopherol content, comprised the accessions of *O. arenaria* Borkh. and *O. purpurea* Jacq. (sect. *Trionychon* Wallr.). All the other accessions of this section presented high δ-tocopherol content. Differences for tocochromanol derivatives within sect. *Trionychon* were paralleled by differences in the fatty acid profile, with the high δ-tocopherol class having also a higher oleic to linoleic acid ratio.

Keywords: *Orobanche*; Orobanchaceae; Broomrape; Chemotaxonomy; Fatty acids; Tocochromanols; Tocopherols; Tocotrienols

1. Introduction

Orobanche L. (Orobanchaceae) is a genus that comprises about 100 species of holoparasitic plants. Beck-Mannagetta (1930) proposed a subgeneric classification of the genus in four sections: *Gymnocaulis* Nutt., *Myzorrhiza* (Phil.) Beck, *Trionychon* Wallr. and *Osproleon* Wallr., the latter known nowadays as sect. *Orobanche* according to the rules of the International Code of Botanical Nomenclature (Greuter, 1988). Although this subgeneric classification is almost universally recognized, there are aspects of *Orobanche* taxonomy that are subject to controversy. For example, Holub (1990) questioned the uniformity of the genus and suggested that it should be splitted into four distinct genera. Also, the taxonomic treatment of many taxa of the genus, especially within the complex groups of *O. ramosa* L., *O. aegyptiaca* Pers., *O. minor* Sutton, and *O. cernua* L. is not satisfactory (Musselman, 1986; Abu Sbaih and Jury, 1994). Current taxonomic classification of *Orobanche* is based on plant morphological characters. Several authors have emphasized the difficulties associated with the exclusive use of morphological traits for *Orobanche* taxonomy (Chater and Webb, 1972; Abu Sbaih and Jury 1994; Musselman, 1994).

Many seed compounds have been used as taxonomic fingerprints in a number of plant families (Gibbs, 1974). Among them, fatty acids have been widely used. Their taxonomic value was already suggested by Earle et al. (1959). More recently, Goffman et al. (1999a) in the Brassicaceae, Velasco and Goffman (1999a) in the Onagraceae and Velasco and Goffman (1999b) in the Boraginaceae demonstrated the taxonomic potential of a combined evaluation of seed fatty acids and tocopherols. Tocopherols, together with tocotrienols, are the compounds exhibiting vitamin E activity. Both types of compounds are known as tocochromanols (Fig. 1). While tocopherols are present in oilseeds, leaves and other green parts of higher plants, tocotrienols are not found in the green parts of the plants but, rather, in the bran and germ fractions of some seeds (Kamal-Eldin and Appelqvist, 1996), vegetable oils and specialized cells like latex tubers (Schultz, 1990).

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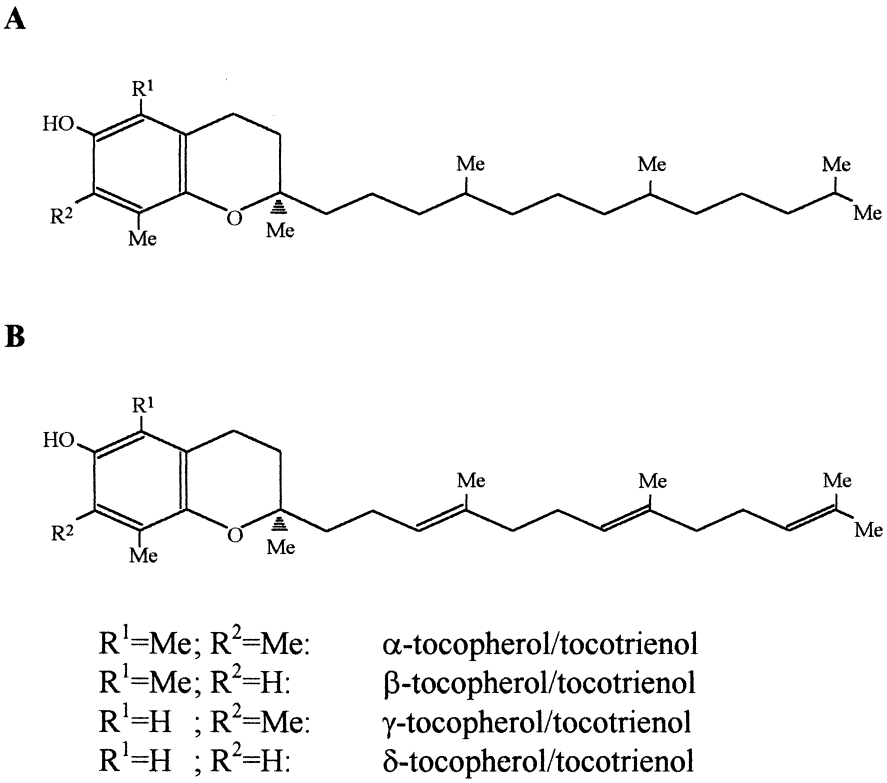

Fig. 1. Chemical structure of tocopherols (A) and tocotrienols (B).

The fatty acid and tocochromanol patterns of *Orobanche* seeds have not been characterized.

The objective of the present study was to evaluate the potential contribution of fatty acids and tocochromanols to the systematics of the genus *Orobanche*.

2. Results and discussion

The fatty acid, tocopherol and tocotrienol patterns of the 49 *Orobanche* accessions are listed in Table 1. The seed oil of the *Orobanche* accessions contained significant amounts of palmitic (16:0), stearic (18:0), oleic (18:1) and linoleic acid (18:2). Two main fatty acid patterns were present; the accessions from most of the species contained predominantly oleic acid, exhibiting a ratio of oleic to linoleic acid above 1.4 (Fig. 2). In contrast, the accessions belonging to *O. arenaria* Borkh., *O. purpurea* Jacq., *O. cumana* Wallr., *O. alsatica* Kirschl., *O. lucorum* A.Braun, *O. rapum-genistae* Thuill., and *O. gracilis* Sm. exhibited a higher linoleic acid content, with a ratio of oleic to linoleic acid below 1.2.

The tocochromanol derivatives α-, β-, γ- and δ-tocopherol, as well as α-, γ- and δ-tocotrienol were detected in the *Orobanche* accessions. The accessions fell into two groups according to the relative contents of tocopherols and tocotrienols. The accessions of sect. *Trionychon* contained predominantly tocopherol derivatives (⩾79% of the total tocochromanol content), whereas the tocotrienol derivatives were more abundant in all the accessions of sect. *Orobanche*, representing more than 91% of the total tocochromanol content. Among the accessions of sect. *Trionychon*, those belonging to *O. arenaria* and *O. purpurea* exhibited a tocochromanol profile dominated by γ-tocopherol (⩾66% of the total tocochromanol content). In contrast, the tocochromanol profile of the accessions from rest of the species of this section was characterized by a high δ-tocopherol content (⩾55% of the total tocochromanol content). Therefore, the tocochromanol pattern divided the accessions into three groups, represented in Fig. 3.

The differences between the sections *Trionychon* and *Orobanche*, established on the basis of morphological traits (Wallroth, 1825), are confirmed at the chemotaxonomic level. Beck-Mannagetta (1890, 1930) established a complex classification within the sect. *Orobanche*, but treated the sect. *Trionychon* as a more homogeneous group. Most authors have followed this uniform treatment of sect. *Trionychon* in subsequent works (e.g., Reuter, 1847; Guimarães,

Table 1
Accession number, taxonomic assignment and accession identification, fatty acid and tocochromanol profiles of 49 *Orobanche* spp. accessions

			Fatty acids[a]				Tocochromanols[b]						
			16:0	18:0	18:1	18:2	α-T	β-T	γ-T	δ-T	α-T3	γ-T3	δ-T3
	Sect. *Trionychon* Wallr.												
1	*O. arenaria* Borkh.	COA 17455	5.1	2.2	39.7	53.0	4.0	0.0	73.6	2.5	0.0	19.9	0.0
2	*O. arenaria* Borkh.	COA 13532	4.7	1.4	42.3	51.6	3.1	0.0	87.3	3.6	0.0	6.1	0.0
3	*O. arenaria* Borkh.	COA 17347	5.9	1.2	45.4	47.5	3.2	0.0	86.6	3.8	0.0	6.4	0.0
4	*O. arenaria* Borkh.	COA 17344	4.4	1.4	39.9	54.4	2.8	0.0	85.5	5.6	0.0	6.2	0.0
5	*O. lavandulacea* Rchb.	COA 25542	6.4	3.7	62.9	27.0	5.8	1.2	25.9	63.0	0.0	0.8	3.3
6	*O. mutelii* F.W. Schultz	COA 25555	8.1	1.4	65.3	25.3	6.2	1.4	24.1	58.6	0.0	3.5	6.2
7	*O. mutelii* F.W. Schultz	COA 17452	5.3	1.0	60.1	33.7	2.1	0.0	23.3	55.1	0.0	6.9	12.7
8	*O. nana* (Reut.) Beck	COA 25556	10.0	1.8	59.7	28.6	7.3	2.4	21.3	69.1	0.0	0.0	0.0
9	*O. purpurea* Jacq.	COA 25416	5.2	1.5	46.8	46.6	5.3	0.0	78.0	1.4	0.0	15.3	0.0
10	*O. ramosa* L.	COA 13605	7.0	3.0	63.7	26.4	1.1	0.0	25.2	67.4	0.0	2.6	3.7
11	*O. ramosa* L.	COA 28895	7.0	2.5	63.6	26.9	0.6	2.6	21.8	69.9	0.0	1.3	3.9
12	*O. ramosa* L.	COA 28896	6.9	3.0	61.2	28.9	7.0	2.8	22.5	57.8	0.0	7.0	2.8
13	*O. ramosa* L.	COA 13798	8.7	1.6	60.2	29.5	2.6	2.6	22.9	68.2	0.0	1.3	2.6
14	*O. schultzii* Mutel	COA 17465	5.5	3.3	67.1	24.1	0.0	0.0	22.5	56.6	0.0	4.7	16.2
15	*O. tunetana* Beck	COA 22550	5.9	2.1	60.0	32.0	2.8	0.0	24.9	62.4	0.0	5.5	4.4
	Sect. *Orobanche* subsect. *Inflatae* Beck grex *Coerulescentes* Beck												
16	*O. cernua* L.	COA 22914	5.3	3.0	58.9	32.9	0.0	0.0	5.4	3.3	0.0	69.6	21.7
17	*O. cernua* L.	COA 24660	5.0	1.8	59.2	34.0	0.0	0.0	3.8	5.0	0.0	77.5	13.8
18	*O. cernua* L.	COA 22113	4.7	1.6	59.4	34.3	0.0	0.0	0.0	1.0	2.1	82.5	14.4
19	*O. cernua* L.	COA 20595	4.8	1.3	56.4	37.5	0.0	0.0	0.0	1.5	0.0	88.4	10.1
20	*O. cernua* L.	COA 20594	4.6	1.0	56.1	38.3	0.0	0.0	0.0	2.1	3.2	75.5	19.2
21	*O. cumana* Wallr.	COA 28295	1.7	1.0	43.4	54.0	0.0	0.0	0.0	0.0	1.0	64.7	34.3
22	*O. cumana* Wallr.	COA 28289	2.5	1.0	39.8	56.7	0.0	0.0	1.7	0.0	3.5	70.7	24.1
23	*O. cumana* Wallr.	COA 22079	2.8	1.1	34.6	61.5	0.0	0.0	2.5	0.0	2.5	84.2	10.8
24	*O. cumana* Wallr.	COA 22080	2.8	1.2	34.4	61.6	0.0	0.0	0.0	0.9	0.0	87.5	11.6
25	*O. cumana* Wallr.	COA 22293	2.6	1.3	33.5	62.6	0.0	0.0	0.0	1.8	1.8	83.6	12.7
26	*O. cumana* Wallr.	COA 17460	4.2	1.5	32.4	61.9	0.0	0.0	0.0	0.0	0.0	86.1	13.9
	Sect. *Orobanche* subsect. *Inflatae* Beck grex *Speciosae* Beck												9
27	*O. crenata* Forssk.	COA 13859	8.4	1.9	60.8	28.9	0.0	0.0	0.0	0.0	0.0	93.8	6.3
28	*O. crenata* Forssk.	COA 13775	8.8	1.8	60.4	29.1	0.0	0.0	0.0	3.7	0.0	90.2	6.1
29	*O. crenata* Forssk.	COA 13671	6.2	1.7	55.3	36.8	0.0	0.0	0.0	0.0	0.0	74.4	25.6
30	*O. crenata* Forssk.	COA 13515	5.7	1.7	55.0	37.7	0.0	0.0	0.0	1.2	0.0	76.5	22.2
	Sect. *Orobanche* subsect. *Angustatae* Beck grex *Minores* Beck												
31	*O. amethystea* Thuill.	COA 13492	9.8	1.4	57.4	31.4	0.0	0.0	0.0	2.8	1.4	87.3	8.5
32	*O. densiflora* Salzm.	COA 28897	9.1	3.1	60.0	27.9	0.0	0.0	0.0	4.1	4.1	61.1	32.7
33	*O. densiflora* Salzm.	COA 13887	11.0	3.7	59.7	25.5	0.0	0.0	0.0	0.8	0.8	69.4	28.9
34	*O. densiflora* Salzm.	COA 13885	9.2	2.7	61.9	26.3	0.0	0.0	0.0	2.4	2.4	72.0	23.2
35	*O. densiflora* Salzm.	COA 25299	10.3	3.1	60.4	26.1	0.0	0.0	0.0	1.6	3.2	64.9	30.3
36	*O. hederae* Duby	COA 28898	7.4	1.7	61.6	29.3	0.0	0.0	0.0	0.0	0.0	75.0	25.0
37	*O. hederae* Duby	COA 28899	6.9	1.6	58.2	33.3	0.0	0.0	0.0	1.5	0.0	68.2	30.3
38	*O. hederae* Duby	COA 28900	6.2	2.0	58.2	33.5	0.0	0.0	0.0	0.0	0.0	73.3	26.7
39	*O. minor* Sutton	COA 25306	8.3	2.5	58.3	31.0	0.0	0.0	0.0	0.0	0.0	90.5	9.5
40	*O. minor* Sutton	COA 28170	7.1	2.6	58.6	31.8	0.0	0.0	0.0	1.7	0.9	79.5	18.0
41	*O. minor* Sutton	COA 25307	8.1	2.2	54.8	34.9	0.0	0.0	0.0	2.7	0.0	75.3	21.9
42	*O. minor* Sutton	COA 13565	7.2	3.1	56.3	33.4	0.0	0.0	0.0	1.1	0.0	85.4	13.5
43	*O. santolinae* Loscos	COA 28901	7.9	2.1	55.1	34.9	0.0	0.0	0.0	0.0	0.0	87.5	12.5
44	*O. santolinae* Loscos	COA 25309	6.0	1.9	60.2	32.0	0.0	0.0	0.0	1.3	2.6	79.2	16.9
	Sect. *Orobanche* subsect. *Angustatae* Beck grex *Curvatae* Beck												
45	*O. alsatica* Kirschl.	COA 28902	7.7	1.2	44.0	47.2	0.0	0.0	0.0	2.3	0.0	86.2	11.5
46	*O. lucorum* A. Braun	COA 28903	4.0	1.5	30.0	64.6	0.0	0.0	0.0	1.8	3.6	83.6	10.9
	Sect. *Orobanche* subsect. *Angustatae* Beck grex *Arcuatae* Beck												
47	*O. rapum-genistae* Thuill.	COA 28904	5.2	1.8	35.6	57.3	0.0	0.0	0.0	7.3	0.0	87.9	4.8
	Sect. *Orobanche* subsect. *Angustatae* Beck grex *Cruentae* Beck												
48	*O. gracilis* Sm.	COA 28905	8.7	2.3	46.5	42.5	0.0	0.0	0.0	0.0	0.0	87.1	11.2
49	*O. foetida* Poir.	COA 17603	8.6	3.2	57.5	30.7	0.0	0.0	0.0	2.5	5.0	85.1	7.4

[a] 16:0, palmitic acid, 18:0, stearic acid, 18:1, oleic acid, 18:2, linoleic acid.
[b] α-T = alpha-, β-T, = beta-, γ-T = gamma-, δ-, δ-T = delta-tocopherol; α-T3 = alpha-, γ-T3 = gamma-, δ-T3 = delta-tocotrienol.

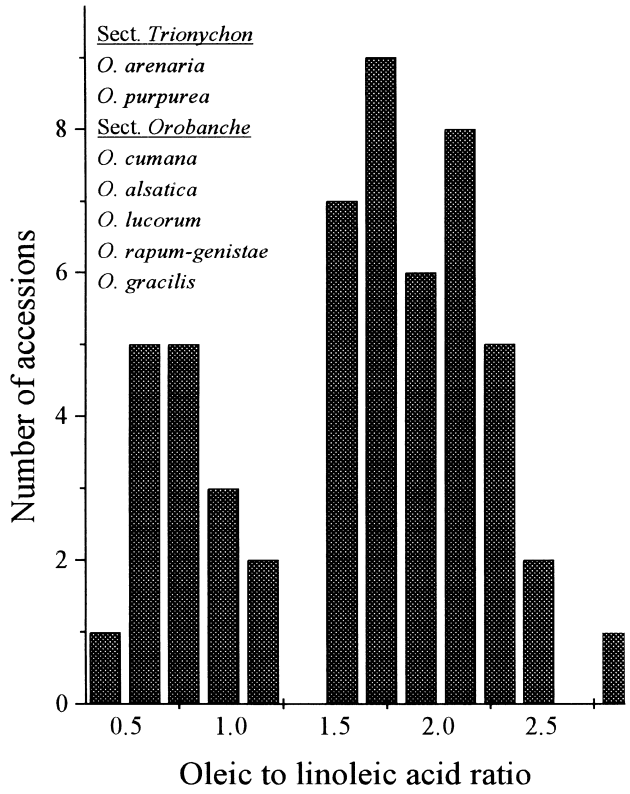

Fig. 2. Histogram of the oleic to linoleic acid ratio in the seed oil of 49 *Orobanche* accessions. The species characterized by low oleic to linoleic acid ratio (≤1.2) are indicated in the figure.

1904; Gilli, 1966; Chater and Webb, 1972; Kreutz, 1995; Uhlich et al., 1995; Pujadas-Salvà and Lora-González, 1996). Conversely, Novopokrovsky and Tzvelev (1958) divided this section into two subsections, *Holoclada* Novopokr. and *Pleioclada* Novopokr. Five of the species of sect. *Trionychon* included in the present evaluation were treated by Novopokrovsky and Tzvelev (1958). Two of them, *O. purpurea* and *O. arenaria*, were classified in subsect. *Holoclada*, whereas the other three, *O. mutelii* F.W. Schultz, *O. nana* (Reut.) Beck and *O. ramosa* L. were assigned to subsect. *Pleioclada*. It is worth noting that the tocochromanol and fatty acid patterns revealed in the present study are in agreement with the above-mentioned classification, i.e., high γ-tocopherol content and low oleic to linoleic acid ratio in *O. arenaria* and *O. purpurea* and high δ-tocopherol and high oleic to linoleic acid ratio in *O. mutelii*, *O. nana* and *O. ramosa* (Table 1, Fig. 3).

The tocochromanol profile was uniform in sect. *Orobanche*, with all the accessions having γ-tocotrienol as the predominant tocochromanol derivative. In consequence, our results evidenced no potential chemotaxonomic value of tocochromanols within this section. In contrast, the accessions of sect. *Orobanche* exhibited variability for the fatty acid profile. The accessions belonging to *O. cumana*, *O. alsatica*, *O. lucorum*, *O. rapum-genistae* and *O. gracilis* had less oleic acid and more linoleic acid than the accessions from rest of the species of this section. It is noteworthy that the accessions of *O. cernua* and *O. cumana*, both considered as conspecific or even synonymous by several authors (e.g., Beck-Mannagetta, 1930; Rechinger, 1943; Chater

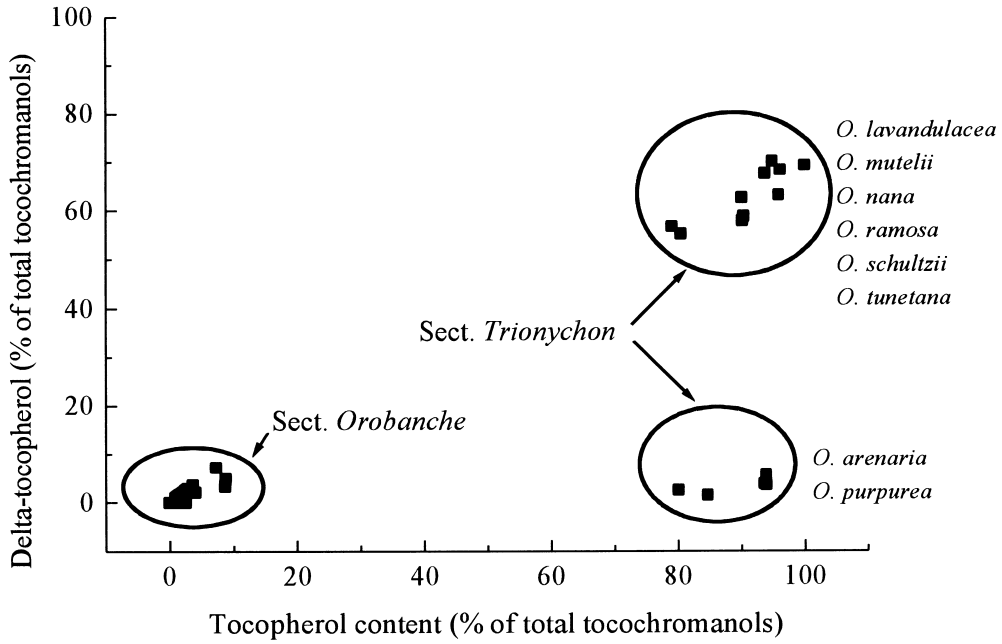

Fig. 3. Scatter plot of tocopherol content (% of the total tocochromanol content) vs. δ-tocopherol content (% of the total tocochromanol content) in 49 accessions of *Orobanche*.

and Webb, 1972), exhibited contrasting seed fatty acid profiles. However, the available information does not allow us to elucidate the taxonomic significance of the fatty acid composition of the seed oil within this section.

Previous studies have revealed the chemotaxonomic value of fatty acid and tocochromanol profiles in several plant families (Goffman et al., 1999; Velasco and Goffman, 1999a, 1999b), which is confirmed for the genus *Orobanche* in the present study. Therefore, the evaluation of fatty acids and tocochromanols in a wider range of species of the Orobanchaceae is suggested as a powerful tool that might contribute to characterize the evolutionary relationships among the species of this family.

3. Experimental

3.1. Plant material

Forty-nine accessions from 21 *Orobanche* species were used for the study. Most of the accessions were collected from the Iberian Peninsula by the senior author. The others were provided by several European botanical gardens. Voucher specimens of all the accessions are deposited at the herbarium COA of the Departmento de Ciencias y Recursos Agrícolas y Forestales of the University of Córdoba, Spain. The corresponding accession numbers are listed in Table 1.

3.2. Fatty acid analyses

About 10 mg seeds were crushed as fine as possible with a stainless steel rod. The resulting powder was transferred into a vial. Fatty acid methyl esters were prepared by simultaneous extraction and methylation following the procedure of Garcés and Mancha (1993), then analysed by gas–liquid chromatography on a Perkin–Elmer Autosystem gas–liquid chromatograph (Perkin-Elmer Corporation, Norwalk, CT, USA) with a 2 m long column packed with 3% SP-2310/2% SP-2300 on Chromosorb WAW (Supelco 1-1833, Bellefonte, PA, USA). A temperature program of 180°C for 10 min, increasing by 3°C min^{-1} up to 210°C maintained for 10 min was used. The injector and flame ionization detector were held at 275 and 250°C, respectively. Fatty acids were identified by comparison of retention times with standards.

3.3. Tocochromanol analyses

Tocochromanol (tocopherol and tocotrienol) patterns were analysed by high-performance liquid chromatography (HPLC) following the procedure of Goffman et al. (1999b). About 5 mg seeds were placed into a 1-ml test tube and crushed as fine as possible with a small stainless steel rod. Tocochromanols were extracted with 500 μl iso-octane for 15 min. After centrifugation, 25 μl of the filtered extract were analysed by HPLC with a 25 cm × 3 mm LiChrospher 100 Diol 5 μm (CS-Chromatographie-Service GmbH, Langerwehe, Germany) column and fluorescence detector (Shimadzu HPLC Monitor RF-1001, Ex: 295 nm, Em: 320 nm). Tocochromanol derivatives were identified by comparison of retention times with standards.

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