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TOCOCHROMANOLS, PLASTOQUINONE AND POLYPRENOLS IN SELECTED PLANT SPECIES FROM CHILEAN PATAGONIA

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A collection of 14 species of Patagonian plants was evaluated for tocopherol content and composition, plastochromanol, plastoquinone content and polyprenol composition. Total tocopherols varied from 35.77 μg/g in *Mutisia decurrens* to 798 μg/g in *Philesia magellanica*. In most cases tocopherol composition was dominated by α-tocopherol, which accounted for more than 90% of total tocopherols. Of all the investigated species only *Misodendrum punctulatum* showed higher content of γ- than α-tocopherol, which is unusual for mature leaves. Plastochromanol, a homologue of γ-tocotrienol, was found in leaves of 10 of the 14 examined species, and was highest in *Araucaria araucana* leaves (132 μg/g). Total content of PQ-9 (oxidized and reduced) was highest in *Fuchsia magellanica* (774.3 μg/g), *Philesia magellanica* (791 μg/g), *Misodendrum linearifolium* (569 μg/g) and *Amomyrtus luma* (518.5 μg/g). Analysis of polyprenol content in the leaves of investigated plant species revealed detectable amounts (>10 μg/g d.w.) of polyprenyl esters in six of them. Accumulation of free polyprenols was detected only in *Chusquea quila* leaves. Selected organs of *Philesia magellanica* and *Fuchsia magellanica* were further quantitatively analyzed for tocochromanol and polyprenol content. With the methods applied, different patterns of the analyzed compounds were identified in all the samples studied. Our results reveal some trends that may be of taxonomic interest. Some of these species can serve as a rich source of such bioactive compounds as tocochromanols or polyprenols.

Key words: Chromatography, HPLC, Patagonian plants, secondary metabolites, tocopherols, plastochromanol, plastoquinone, polyprenols.

INTRODUCTION

Patagonia is the southernmost geographic region of South America, located mostly in Argentina and Chile. Due to wide fluctuations in temperature, altitude and precipitation, Patagonia hosts a variety of vegetation types. The southwestern coast is covered by Magellanic moorland with characteristic dwarf shrubs. The area near the icefields is overgrown by evergreen temperate rain forest (Magellanic subpolar forests) of southern beech (*Nothofagus betuloides*) (McEwan et al., 1997). The region where precipitation is lower has deciduous woodland rain forest with *Notofagus pumilo, Berberis* sp. and *Gunnera magellanica* as dominant species. East of the mountains is broad steppe with low shrubs and small perennials. Above this zone there is rain forest also (McEwan et al., 1997). In most cases the species analyzed in this study were collected in evergreen rainforest in Puyuhuapi 44°21'72°34'.

As part of our current work on wild Patagonian flora, here we report the results of a study on selected prenyllipids in the leaves of 14 species growing within the Chilean part of Patagonia. We characterized representatives of four classes: *Polypodiophyta, Bryophyta, Pinophyta* and *Magnoliophyta*. Using different chromatographic techniques we isolated and characterized compounds including α-tocopherol, γ-tocopherol, δ-tocopherol, plastochromanol, oxidized and reduced forms of plastoquinone, polyprenols and their esters.

Tocopherols are a group of four (α, β, γ, δ) lipophilic antioxidants synthesized by photosynthetic organisms, occurring mainly in leaves and seeds

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Abbreviations: DW – dry weight; HPLC – high-performance liquid chromatography; TLC – thin layer chromatography; PC – plastochromanol; PQ – plastoquinone; PQH_2 – reduced plastoquinone.

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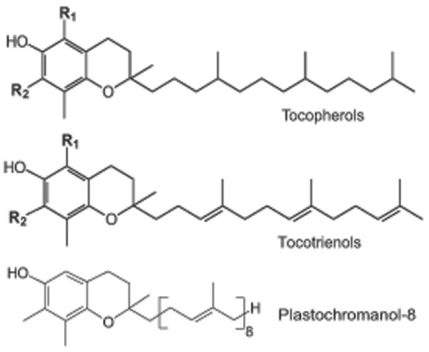
(Munné-Bosch and Alegre, 2002). They are amphipathic molecules showing vitamin E activity. Tocopherols are members of the tocochromanols, in which the molecule contains a polar chromanol ring ("head") and a long prenoid side-chain ("tail") (Munné-Bosch and Alegre, 2002; Kumar et al., 2005). The general structure of tocochromanols is shown in Figure 1. Depending on the degree of saturation of the hydrophobic tail, two types of tocochromanol compounds are distinguished: tocopherols (with a fully saturated 16-carbon phytol side-chain), and tocotrienols (containing a 16-carbon side-chain with 3 double bonds in positions C-3, C-7 and C-11). Each group encompasses four isomers (α, β-, γ-, δ-) which differ in the number and position of methyl groups in the chromanol head group (Munné-Bosch and Alegre, 2002). Another lipophilic antioxidant, plastochromanol-8 (PC), also belongs to the group of tocochromanols. PC is a γ-tocotrienol homologue and also shows antioxidant activity (Olejnik et al., 1997). The only difference between these two compounds is a longer prenyl chain in PC-8 (8 isoprenoid subunits) (Fig. 1). PC has been found in vegetable oils (Ahmed et al., 2005), mainly in the oil of *Brassica napus*, *Linum* sp. and *Cannabis sativa* seeds (Olejnik et al., 1997; Kriese et al., 2004; Gruszka and Kruk, 2007). Its occurrence in leaves has not been thoroughly examined yet. Tocochromanols act as antioxidants, and this function is attributed mainly to inhibition of membrane lipid peroxidation and to scavenging of reactive oxygen species (Munné-Bosch and Alegre, 2002; Trebst et al., 2002; Kruk et al., 2005), but other functions have also been shown in plant metabolism, such as a role in sugar export from leaves to phloem (Porfirova et al., 2002; Hofius et al., 2004). The literature data on the content and composition of tocopherols have focused on seed oils because of the nutritional importance of vitamin E. The reported concentration of tocopherols in seed oils reaches up to 2 mg/g oil (DellaPenna, 2005), while in leaves it is usually significantly lower (10–50 μg/g fresh weight) with a few exceptions: content of 300–500 μg/g fresh weight in palm oil (Grusak and DellaPenna, 1999) and a few tropical plants (Ching and Mohamed, 2001), or nearly 1 mg/g fresh weight in *Eucalyptus gunni* (El Kayal et al., 2006). With respect to isomer composition, seed oils are usually dominated by γ-tocopherol, while leaves are rich in α-tocopherol (>90%) (DellaPenna, 2005; Lichtenthaler, 2007; Szymańska and Kruk, 2008a) with only a few exceptions reported for lettuce (DellaPenna, 2005; Szymańska and Kruk 2008a) or parasitic dodder shoots (van der Kooij et al., 2005; Szymańska and Kruk, 2008a) in which the highest levels of γ or δ-tocopherol were found. The meaning of these differences is so far unknown.

Plastoquinone (PQ) is known mainly as a component of the electron transport chain in photosynthesis, but its reduced form (PQH_2), similarly to tocopherols,



Tocopherol/ Tocotrienol	R_1	R_2
α	CH_3	CH_3
β	CH_3	H
γ	H	CH_3
δ	H	H

Fig. 1. Chemical structure of tocochromanols.

shows antioxidant activity *in vitro* (Kruk et al., 1994, 1997). It has also been shown that PQH_2 can inhibit membrane lipid peroxidation in vivo (Maciejewska et al., 2002) and scavenge reactive oxygen species (Kruk et al., 2003; Kruk and Trebst, 2008). Numerous data also indicate the regulatory function of the PQ pool in many physiological and molecular processes (Karpinski et al., 1999).

Polyprenols are common constituents of plant photosynthetic tissue. They always accumulate as mixtures of homologues with the notable exception of the all-*trans*-type counterpart (common name solanesol) (Hemming, 1985). Polyprenol chain length has been found to vary over a large range in nature, with polyprenol molecules consisting of from 5–6 up to 100 isoprene residues. The composition of the polyprenol mixture in photosynthetic tissue is considered to be a species-specific chemotaxonomic marker. Polyprenols are found in cells in the form of free alcohols and/or esters with carboxylic acids; a fraction of polyisoprenoid phosphates has also been detected. The biological role of phosphorylated polyisoprenoid alcohols as cofactors in the biosynthesis of glycoproteins and GPI-anchor or bacterial peptidoglycan is well characterized. They are also suggested to serve as donors of isoprenoid groups during protein prenylation. The role of free polyisoprenoid alcohols and carboxylic esters, on the other hand, is uncertain. Biophysical studies have shown that isoprenoids increase the permeability and fluidity of model membranes and also enhance the fusion of membranes. There is also evidence that these compounds are involved in the transport of ER and vacuolar proteins (Swiezewska and Danikiewicz, 2005, and references therein).

We examined the content and composition of tocochromanols, plastoquinol and polyprenols in 14

TABLE 1. Tocopherol content and composition, and plastochromanol content in leaves of selected Patagonian plants. The given values are means±SE. α-T – α-tocopherol, γ-T – γ-tocopherol, δ-T – δ-tocopherol, Tocs – tocopherols; PC – plastochromanol; DW – dry weight

Species	μg α-T/g DW	μg γ-T/g DW	μg δ-T/g DW	Total Tocs μg/g DW	Total PQ ($PQ+PQH_2$) μg/g DW	μg PC/g DW
Amomyrtus luma	190.0±5.7	5.5±0.7	0.0	195.5±6.4	518.5±28	45.0±4.5
Araucaria araucana	218.0±14	9.2±1.1	0.0	227.2±15.1	156.0±9.8	132.0±8.4
Berberis buxifolia	290.0±11.2	5.8±2.3	0.9±0.01	296.7±13.5	268.0±13.5	9.0±1.3
Blechnum chilense	56.3±1.6	1.9±0.4	1.1±0.07	59.3±2.0	83.0±9.6	0.0
Chusquea quila	123.0±9.3	3.7±1.7	0.0	126.7±11.0	380.0±8.7	32.0±1.1
Fuchsia magellanica	116.0±4	3.9±0.9	0.0	119.9±4.9	774.3±32.5	66.0±3.3
Hypopterygium arbuscula	37.0±2.8	1.0±0.06	0.0	38.0±2.9	23.3±3.7	3.4±0.7
Misodendrum punctulatum	277.0±11	322.8±9.5	55.0±4.7	654.8±25.2	294.0±21	0.0
Misodendrum linearifolium	198.0±4.2	2.8±0.3	0.0	200.8±4.5	569.0±30.4	23.6±2.7
Mitraria coccinea	54.0±2	15.0±0.6	0.0	69.0±2.6	347.0±21.5	11.5±1.8
Mutisia decurrens	20.0±1.2	12.0±0.8	3.8±0.7	35.8±2.7	190.0±8.3	0.0
Mutisia spinosa	30.0±2.5	12.0±1.2	0.0	42.0±3.7	148.0±1.3	2.8±0.2
Notophagus betuloides	99.0±8	4.4±0.9	0.14±0.008	103.5±8.9	125.0±3.7	44.0± .6
Philesia magellanica	470.0±21	308.0±13	20.0±4.5	798.0±38.5	791.0±39.5	0.0

selected Patagonian plant species. The variability of such compounds has not been studied in these plants. In this work we assessed the variability of tocopherols, plastochromanol, polyprenols and polyprenyl esters in these species.

MATERIALS AND METHODS

Leaves of *Amomyrtus luma*, *Araucaria araucana*, *Berberis microphylla, Blechnum chilense, Chusquea quila, Fuchsia magellanica, Hypopterygium arbuscula, Misodendrum linearifolium, Misodendrum punctulatum, Mitraria coccinea, Mutisia decurrens, Mutisia spinosa, Nothofagus betuloides* and *Philesia magellanica*, and flowers and fruits of *Fuchsia magellanica* and *Philesia magellanica* were collected from natural stands in the Chilean part of Patagonia (Puyuhuapi, 44°21'S, 72°34'W) during Southern Hemisphere summer. They were identified by Dr. Roberto Rodríguez of the Department of Botany, Faculty of Natural and Oceanographic Sciences, University of Concepción, Chile, and voucher specimens were deposited in its herbarium (Universidad de Concepción Herbario).

For tocopherol analysis, 60–150 mg samples of leaves, flowers or fruits were ground in a mortar with 1.5 ml cold HPLC solvent (acetonitrile/methanol/water, 72:8:1 v/v/v). The extract was transferred to an Eppendorf tube, briefly centrifuged (60 s) on a benchtop centrifuge (10,000 g) and analyzed by HPLC. Plastochromanol and plastoquinone analysis employed the same method but with a different HPLC solvent (methanol/hexane, 340:20 v/v). The HPLC measurements were performed using a 100 μl loop, Jasco PU-980 pump and UV-VIS detector system UV-970, Shimadzu RF10-AXL fluorescence detector (excitation/emission detection at 290/330 nm), Teknokroma (Barcelona, Spain) C18 reverse-phase column (Nucleosil 100, 250 × 4 mm, 5 μm) and isocratic solvent system (acetonitrile/methanol/water, 72:8:1 v/v/v) at a flow rate of 1.5 ml/min. Tocopherol homologues of HPLC grade (≥99.5%) were purchased from Merck. PQ-9 was obtained as previously described (Kruk, 1988) and PC was obtained according to the procedure described by Gruszka and Kruk (2007).

For semiquantitative analysis of polyisoprenoids, dried leaves (300–500 mg) were homogenized with an UltraTurrax homogenizer at top speed in 4 ml acetone/hexane (1:1, v/v). After 24 h the extracts were subjected to thin-layer chromatography (TLC) on Silica gel plates in ethyl acetate/toluene (1:9, v/v) in the presence of standards (mixture of polyprenyl esters from *Ginkgo biloba* and free polyprenol alcohols from *Magnolia kobus*). The locations of polyprenols were detected with iodine vapor.

Quantitative analysis of polyprenols was performed as described earlier (Skorupinska-Tudek, 2003).

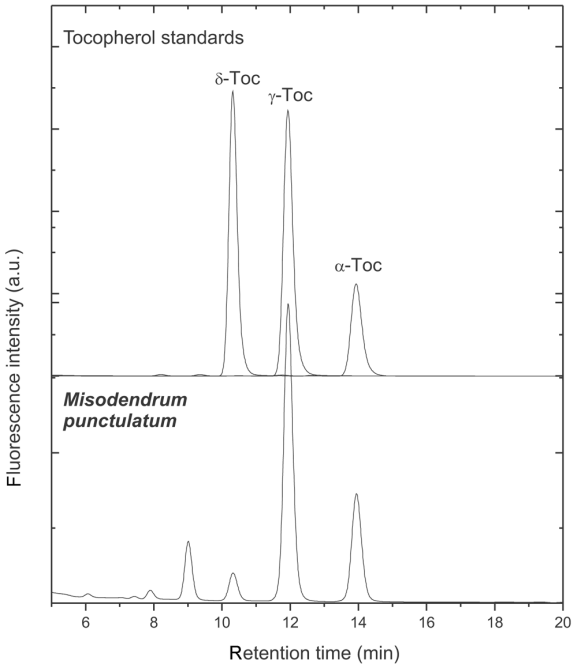


Fig. 2. HPLC chromatograms of tocopherol standards (10 μM each) and extracts of *Misodendrum punctulatum*. Details given in the text.

RESULTS AND DISCUSSION

Table 1 presents the content of α-, γ- and δ-tocopherol, total tocopherol content and plastochromanol content in the 14 Patagonian species. Total tocopherols ranged from 35.77 μg/g in *Mutisia decurrens* to 798 μg/g in *Philesia magellanica*. Total tocopherols were high in *Berberis buxifolia* (296.7 μg/g), *Araucaria araucana* (227.2 μg/g), *Misodendrum linearifolium* (200.85 μg/g) and *Amomyrtus luma* (195.54 μg/g). With one exception, tocopherol composition was dominated by α-tocopherol, which accounted for more than 90% of total tocopherols. It was highest in leaves of the shrub *Philesia magellanica* (470 μg/g) (Tab. 1), and lowest in *Mutisia decurrens* (20 μg/g). All the investigated species contained γ-tocopherol, but its level as well as the level of α-tocopherol varied widely. The richest sources of γ-homologue were leaves of *Misodendrum punctulatum* (322.8 μg/g) and *Philesia magellanica* (308 μg/g). The tocopherol composition in *Misodendrum punctulatum*, with γ-tocopherol higher than α-tocopherol, was quite unusual (Fig. 2). Such a composition is typical of seeds and etiolated leaves, not of mature leaves (Szymańska and Kruk, 2008b). A much higher level of γ- than α-tocopherol has recently been found in primary runner bean leaves (Szymanska and Kruk, 2008b). In that work, γ-tocopherol was suggested to play a role in drought stress resistance. The high level of γ-tocopherol in *Misodendrum punctulatum* possibly takes part in the plant response to environmental conditions. Desmarchelier et al. (2005) showed that a dry methanol extract of *Misodendrum punctulatum* efficiently scavenged free radicals and also displayed antioxidant activity. It is well established that *Misodendrum punctulatum* contains bioactive compounds including myzodendrone, catechin and zingerone, which are highly effective free radical scavengers (Reyes et al., 1986). This group of bioactive compounds needs to be extended to tocochromanols.

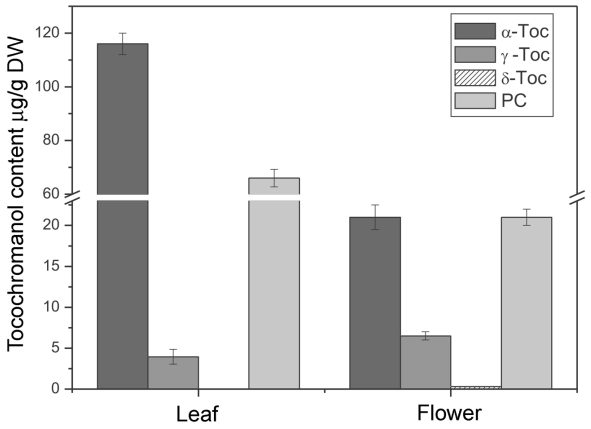


Fig. 3. Tocochromanol content in leaves and flowers of *Fuchsia magellanica*.

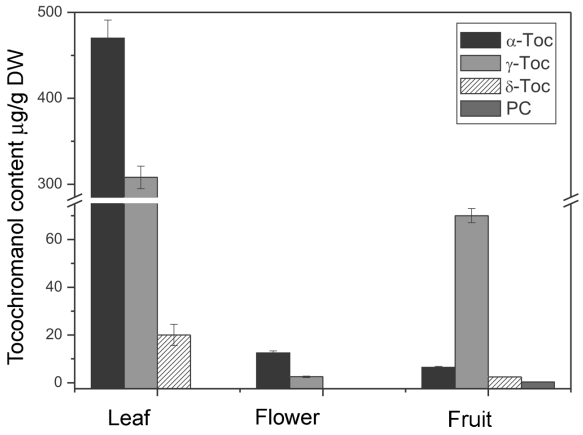


Fig. 4. Tocochromanol content in leaves, flowers and fruits of *Philesia magellanica*.

Six of the 14 investigated species were found to contain δ-tocopherol (Tab. 1). Its content was highest in *Misodendrum punctulatum* (55 μg/g). Surprisingly, *Misodendrum linearifolium*, though it belongs to the same genus as *M. punctulatum*, had no δ-tocopherol at all and had more than 100-fold lower content of γ-tocopherol. These trends may be of taxonomic interest.

Leaves of 10 of the 14 species showed the presence of plastochromanol (PC). Its level ranged from 3.4 μg/g in *Hypopterygium arbuscula* to 132 μg/g in *Araucaria araucana*. PC was originally thought to be

TABLE 2. Polyprenol content in organ tissues of selected Patagonian plants

Species	Tissue	Polyprenol	
		Pattern	Total content µg/g d.w.
Philesia magellanica	Leaves	Pren- 13, **14**, 15, 16, 17, 18, 19, 20	27.0 ± 5.2
	Flowers	Pren- 13, **14**, **15**,**16**, 17, 18, 19, **20**, 21, 22	13.5 ± 2.8
	Fruits	Pren- 13, **14**, **15**,**16**, 17, 18, 19, **20**, 21, 22	15.9 ± 3.3
Fuchsia magellanica	Leaves	Pren-13, 14, **15**, 16, 17, 18	34.1 ± 4.2
	Flowers	Pren-13, 14, **15**, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25	24.6 ± 3.1

restricted to seed oils. In seed oils PC has an antioxidant function, protecting polyunsaturated fatty acids from oxidation (Olejnik et al., 1997). Intensive analysis revealed its presence also in leaves, and its level did not increase under high light but changed with leaf age, suggesting that its role is connected rather with senescence (Szymańska and Kruk, 2008c). The high level of PC in evergreen *Araucaria araucana* may constitute support for that view, owing to the longevity of its leaves, and this may be related to the medical use of this species (Montes and Wilkomirsky, 1987).

The composition and content of tocochromanols (α-, γ-, δ-tocopherol and plastochromanol) varied widely in leaves and flowers of *Fuchsia magellanica* and in leaves, flowers and fruits of *Philesia magellanica*. In *Fuchsia magellanica*, total tocochromanols were 3.6-fold higher in leaves than in flowers (Fig. 3). In leaves α-tocopherol clearly dominated, unlike in flowers, where α-tocopherol and PC occurred at equal levels. δ-Tocopherol was present in *Fuchsia* flowers but absent in leaves. In view of the absence of photosynthetic apparatus in flowers, the occurrence of tocopherols and PC in these organs suggests non-antioxidant functions, such as a role in sugar transport (Porfirova et al., 2002) or intracellular signaling (Munné-Bosch and Falk, 2004). *Philesia magellanica* showed the highest content of tocopherols in leaves of all the investigated species (Tab. 1). Levels of tocopherols and plastochromanol were also measured in flowers and fruits (Fig. 4). Though not as high as in leaves, the presence of these compounds in non-green parts confirms our previous ideas (Szymańska and Kruk, 2008b). In flowers only α- and γ-tocopherol were detected; in fruits all four components were found (Fig. 4). Total tocochromanols in flowers was about 50-fold lower than in leaves, and in fruits nearly 10-fold lower. Interestingly, unlike the fruits, flowers possessed no δ-tocopherol or PC. These differences are probably connected with the stage of development of these species and also the different functions of the examined organs.

Plastoquinone-9 (PQ-9) content showed pronounced differences between the investigated species (Tab. 1). Total PQ-9, including its oxidized and reduced forms, was very high in four species: *Fuchsia magellanica* (774.3 µg/g) and *Philesia magellanica* (791 µg/g), *Misodendrum linearifolium* (569 µg/g) and *Amomyrtus luma* (518.5 µg/g). Two species showed very low total PQ-9: *Blechnum chilense* (83 µg/g) and *Hypopterygium arbuscula* (23.3 µg/g). Accumulation of PQ-9 is usually connected with tissue aging. This may have been the case in our study. Reduced PQ-9 was detected in all the investigated species except one, *Mutisia spinosa* (Tab. 1).

Semiquantitative analysis of polyprenol content in the leaves of several plant species revealed detectable amounts (> 10 µg/g DW) of polyprenyl esters in *Philesia magellanica*, *Fuchsia magellanica*, *Misodendrum punctulatum*, *Chusquea quila*, *Mutisia decurrens* and *Misodendrum linearifolium*. Accumulation of free polyprenols was detected only in leaves of *Chusquea quila*. Selected organs of *Philesia magellanica* and *Fuchsia magellanica* were further quantitatively analyzed for polyprenol content (Tab. 2). The HPLC/UV method identified the families of polyisoprenoid homologues in all the samples studied. According to their chromatographic properties these polyisoprenoid alcohols were tentatively identified as polyprenols, although further analyses (HPLC/MS and NMR) are needed for their final structural identification. The leaves of both analyzed plants contained single families of 'middle-length' polyprenols, with Pren-15 dominating. Interestingly, a two-family pattern of polyprenols was detected in the flowers and fruits of *Philesia magellanica* (Pren-16 and -20 dominating). This phenomenon – different polyprenol compositions in the organs of the same plant species – has been observed previously (Skorupińska-Tudek, 2003). The highest content of polyprenols, exceeding 30 µg/g DW, was in leaves of *Fuchsia magellanica*.

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