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Phenolic Diterpenes, Flavones, and Rosmarinic Acid Distribution during the Development of Leaves, Flowers, Stems, and Roots of *Rosmarinus officinalis*. Antioxidant Activity

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The distribution of six compounds with three different polyphenol skeletons have been studied in *Rosmarinus officinalis*: phenolic diterpenes (carnosic acid, carnosol, and 12-*O*-methylcarnosic acid), caffeoyl derivatives (rosmarinic acid), and flavones (isoscutellarein 7-*O*-glucoside and genkwanin), each showing a characteristic behavior and distribution during the vegetative cycle. Only in leaves were all six compounds present, and the highest accumulation rate was related with the young stages of development. Rosmarinic acid showed the highest concentrations of all the polyphenols in all organs. The distribution of this acid in leaves, flowers, and stems suggests that in the first stages of flower growth, levels were due to in situ biosynthesis, and in the last stages, the contribution of transport phenomena was increased. The antioxidant activity of six extracts with different polyphenolic composition was evaluated in aqueous and lipid systems. The results clearly suggest that rosemary extracts are excellent antioxidants in both aqueous and lipid systems.

KEYWORDS: ***Rosmarinus officinalis*; carnosic acid; carnosol; 12-*O*-methylcarnosic acid; diterpene phenols; genkwanin; isoscutellarein 7-*O*-glucoside; rosmarinic acid; antioxidant**

INTRODUCTION

A large number of polyphenolic compounds with antioxidant activity have been identified in *Rosmarinus officinalis*; the presence of phenolic diterpenes such as carnosic acid (CA) and carnosol (*1*−*4*) has been reported. Carnosic acid (1) is degradated in polar solvents and appears to be oxidized into other phenolic compounds such as carnosol (2), rosmanol, epirosmanol, 7-methylepirosmanol, and methyl carnosate (*1*, *5*−*8*) (**Figure 1**). In addition, several flavonoids, such as genkwanin (5), hispidulin 7-*O*-glucoside, cirsimaritin, luteolin, and isoscutellarein 7-*O*-glucoside (6), are found in Labiatae plants (*7*, *9*), where the phenolic compounds and rosmarinic (RO) (4) and caffeic acids are also present (*4*, *10*, *11*).

The antioxidant activity of rosemary extracts depends on their phenolic composition. There are many reports of the antioxidant activity of rosemary extracts and their compounds, determined by various methods in different lipid and aqueous systems. In lipid systems, extracts with higher phenolic diterpene content are more effective (*12*), while in aqueous systems, rosmarinic acid exhibits the highest antioxidant activity (*4*, *11*). In addition, rosemary extracts are used in medicine as antiinflammatory (*13*) and antimicrobial agents (*14*).

In most studies concerning antioxidant activity, purified extracts are used (*1*, *3*, *4*, *12*). Occasionally, commercially available extracts have been used, but their active compounds have only been partially quantified (*15*). Very few reports have been published on the distribution of phenolic diterpenes during the growth and vegetative development of rosemary leaves (*16*−*18*) and different organs at the subcellular level (*18*).

The aims of this study were as follows: to identify and characterize the most abundant polyphenol compounds in rosemary plants (carnosic acid, carnosol, 12-*O*-methylcarnosic acid (phenolic diterpenes), rosmarinic acid (caffeoyl compound), genkwanin and isoscutellarein 7-*O*-glucoside (flavones)); to study the quantitative distribution of these polyphenols during the development of the different plant organs by means of HPLC methods using a diode array detector, combining and modifying other previously described methods (*2*, *10*, *19*); and to evaluate the antioxidant activity of the different extracts in aqueous and lipid systems, using ascorbic acid and α-tocopherol as references.

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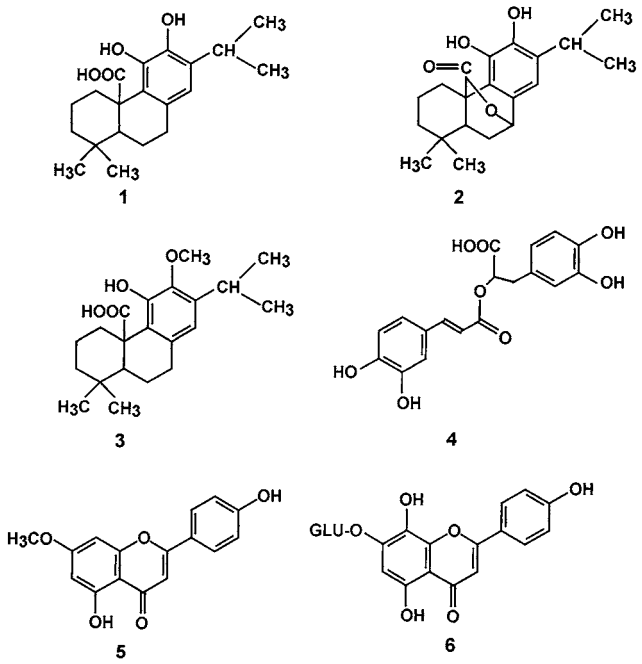

Figure 1. Chemical structures of the compounds studied: carnosic acid (**1**), carnosol (**2**), 12-*O*-methylcarnosic acid (**3**), rosmarinic acid (**4**), genkwanin (**5**) and isoscutellarein 7-*O*-glucoside (**6**).

MATERIALS AND METHODS

Plant Materials. Rosemary leaves, flowers, stems, and roots were obtained from three month old *R. officinalis* plants (initiation cycle) grown in greenhouses at the University of Murcia. The plant materials were collected between February 2001 and February 2002, immediately dried at 40 °C, and ground for extraction and analysis.

Materials and Reagents. Carnosic acid and carnosol were obtained from the R&D Department of Flavex Naturextrakte GmbH (Rehlingen, Germany) and the Institute of Pharmacognosy and Analytical Phytochemistry of the University of Saarebruck (Germany). Genkwanin and rosmarinic acid were obtained from Extrasynthèse (Genay, France). Isoscutellarein 7-*O*-glucoside and 12-*O*-methylcarnosic acid were obtained by HPLC semipreparative methods in the Plant Physiology Department at the University of Murcia.

Linoleic acid, α-tocopherol, and lard were obtained from Sigma Chemical Co. (Madrid, Spain). HPLC grade ethanol, methanol, dimethyl sulfoxide (DMSO), acetone and water, trichloroacetic acid (TCA), and 2-thiobarbituric acid were obtained from Merck (Darmstadt, Germany). L-ascorbic acid was obtained from Panreac (Madrid, Spain).

Extraction of Polyphenols from Plant Materials for Analytical Purposes. The polyphenols were extracted for analytical chromatography from previously dried plant materials, using DMSO in the ratio of 20 mg/mL. The measurements were performed on six plants, and the values obtained at each age were used to express the distribution of polyphenols in leaves, flowers, stems, and roots (% dry weight). All the solutions were filtered through a 0.45-μm nylon membrane.

Obtaining Rosemary Extracts. Figure 2 shows the complete scheme for obtaining different rosemary extracts. The extractions were made for 1 h at room temperature. To obtain the CA 2 dry powder, the CA 1 powder was washed with methanol/water (3:1), as shown in **Figure 2**. After filtration, the clarified solution was concentrated to a dry powder under vacuum at 40 °C. Similar steps were used to obtain the subsequent dry powders, using the solvents shown in **Figure 2**. All the concentration and the powder drying steps were made under vacuum at 40 °C.

Chromatographic Analysis. Two HPLC methods were developed for the identification and quantification of the polyphenols in plant materials and the rosemary extracts. The HPLC equipment used was a Hewlett-Packard HP 1100 equipped with a diode array detector. The stationary phase was a 250 × 4 mm i.d, 5 μm, C_{18} LiChrospher 100 analytical column (Merck, Darmstadt, Germany) thermostated at 30 °C. The flow rate was 1 mL/min, and the elution was monitored at 280 nm. The mobile phases for chromatographic analysis of phenolic diterpenes and flavonoids (method 1) were (A) water with 1% acetic acid and (B) methanol. An isocratic step of 50% B during 5 min was run, followed by a linear gradient to 100% B in 25 min, maintaining this composition for 10 min; the system was then reequilibrated to initial composition in 5 min.

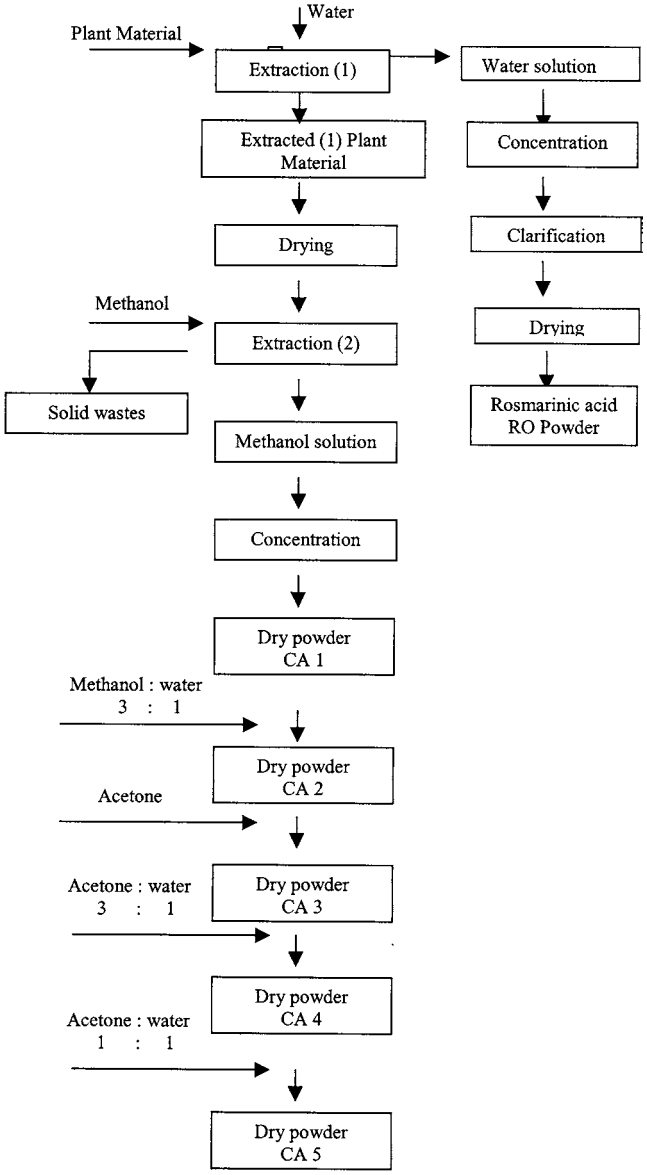

Figure 2. Extraction scheme of six different rosemary extracts: RO, water-soluble fraction where the main compound is rosmarinic acid; CA 1, crude methanolic extract where the main compound is carnosic acid; CA 2, purified methanolic extract; CA 3, acetone extract from purified methanolic extract; CA 4, purified acetone extract; and CA 5, final highest purified carnosic acid extract. These powders were used in antioxidant assays.

The mobile phases for chromatographic analysis of rosmarinic acid (method 2) were: (A) water with 2.5% acetic acid and (B) acetonitrile. A solution of 95% A and 5% B was maintained for 20 min and then changed to 50% A in 20 min; this was then changed to 20% A in 10 min, and reequilibrated in 10 min to the initial composition. Phenolic compounds were identified by comparison of their retention times with the corresponding standards and by their UV spectra, obtained with the diode array detector.

Isolation of Polyphenols. For the isolation of the main polyphenols (**1**, carnosic acid; **2**, carnosol; **3**, 12-*O*-methylcarnosic acid; **4**, rosmarinic acid; **5**, genkwanin, and **6**, isoscutellarein 7-*O*-glucoside), a 250 × 10 mm i.d., 5 μm, C_{18} semipreparative column was used, with a flow rate

of 3 mL/min. The mobile phases and the gradients used were the same as for polyphenol identifications. The fractions were collected with a Pharmacia FRAC 100 (Pharmacia LKB Biotechnology, Uppsala, Sweden). For this process, 50 g of leaves were extracted with DMSO in the ratio of 200 mg/mL. The solutions were filtered through a 0.45-μm nylon membrane.

Thiobarbituric (TBA) Method: Autoxidation of Linoleic Acid. A 2 g portion of linoleic acid was solubilized in 50 mL of ethanol, and then 100 mL of 0.1 M phosphate buffer, pH 7.0, and 50 mL of water were added. Finally, the solution of the rosemary extracts (RO and CA powders) or L-ascorbic acid in 50 mL of ethanol was added, to a final concentration of 200 ppm. The reference solution was prepared by the addition of 50 mL of ethanol. Oxidation was performed by letting in atmospheric air at 25 °C. To detect the malonyldialdehyde formed, 5 mL of trichloroacetic acid (TCA) was added to 2.5 mL of oxidized solution to stop the oxidation, and then 2.5 mL of TBA (0.67% in water) was added. The reaction mixture was shaken and incubated in a boiling water bath for 15 min. The mixture was cooled and centrifuged at 1200 rpm for 20 min. The final color developed was measured at 532 nm, using a Perkin-Elmer UV/Vis spectrophotometer. The protection factor, also called the activity index, of the antioxidant is defined as the ratio of the induction times measured with and without antioxidant and can be taken as a measure of the antioxidant efficacy.

Rancimat Test: Determination of Induction Period. The widely used rancimat test, which is generally accepted as a measured of antioxidant efficacy (*2*, *3*, *20*), is based on an accelerated aging of the lipophilic target material, in this case 3.5 g of lard. This is achieved by heating the lard to a high temperature as air is allowed to flow through the material. The studied extracts and α-tocopherol were added to a final concentration of 500 ppm. A temperature of 120 °C and an air flow of 15 L/h were chosen as standard conditions. The oxidative stress was tolerated for a certain period of time (the induction time). This was followed by a rapid autocatalytic breakdown of the lipid test material, which is accompanied by the formation of short chain fatty acids. These were transferred by means of the air stream into a conductivity measuring cell containing distilled water. In this way, a sharp increase in conductivity was detected by the Rancimat 679 equipment (Metrohm, CH.Herisau, Switzerland) used. The protection factor, also called the activity index, of the antioxidant is defined as the ratio of the induction times measured with and without antioxidant and can be taken as a measure of the antioxidant efficacy.

RESULTS

Identification of the Main Polyphenols in Leaves, Flowers, Stems, and Roots of *Rosmarinus officinalis.* HPLC analysis of DMSO extracts of leaves, flowers, stems, and roots of *R. officinalis* during growth indicated the presence of many polyphenols. **Figure 3A** shows a characteristic chromatogram of an extract from *R. officinalis* leaves, an organ that contains all the compounds studied. The peaks whose retention times were identical to those of carnosic acid (**1**), carnosol (**2**), 12-*O*-methylcarnosic acid (**3**), rosmarinic acid (**4**), genkwanin (**5**), and isoscutellarein 7-*O*-glucoside (**6**) are numbered. The chromatograms obtained for flower, stem, and root extracts are similar, although the relative proportions of each compound differ, and peaks 5 and 6 are absent from the flower, stem and root extracts, and peak 3 from stems and roots. Another chromatographic method was developed to obtain a better elucidation and quantification of rosmarinic acid **(Figure 3B)**.

Compounds **1**−**6** had IR and ^{1}H NMR spectra identical with those of carnosic acid, carnosol, 12-*O*-methylcarnosic acid, rosmarinic acid, genkwanin, and isoscutellarein 7-*O*-glucoside (*2*, *5*, *7*, *17*, *21*).

Changes in the Levels of Phenolic Diterpenes (Carnosic Acid, Carnosol, and 12-*O*-Methylcarnosic Acid) in *Rosmarinus officinalis* Leaves, Flowers, Stems, and Roots during Plant Maturation. Growth of rosemary leaves, as measured by leaf length, occurs during the first month of the vegetative cycle and is practically sigmoid, showing 10−15 mm length in 8−10 day-old leaves and reaching its maximum size, 25−30 mm length, at approximately 30−35 days. After this time, the leaf size remains constant. **Figure 4** shows the changes in the content of carnosic acid during the vegetative development of leaves, flowers, and stems (% dry weight) (2001−2002 season). In leaves, the concentration of carnosic acid increased during the first stages of growth and reached its maximum when the leaves measured 10−15 mm in length. Thereafter, the levels of this diterpene decreased until leaf development finished (25−30 mm). The concentration remained practically constant until flowering, increasing slightly during summer. At this point, the concentration in leaves decreased but increased in stems and flowers. The levels of this acid in flowers increased slightly as the flower developed and reached similar levels to those in leaves. In stems (vascular system), the concentration was lower than in leaves and flowers but qualitatively very significant. The concentration increased when flowers were present on plants, and the maximum levels were maintained during the first stages of leaf growth, after which carnosic acid practically disappeared.

Carnosol showed a similar behavior to carnosic acid during the first stages of leaf growth, with maximum levels being reached at 5−10 mm. Then, between March and June, the carnosol content of leaves slowly increased. When flowers were present (July−February) the concentration in leaves decreased as it increased in flowers and stems.

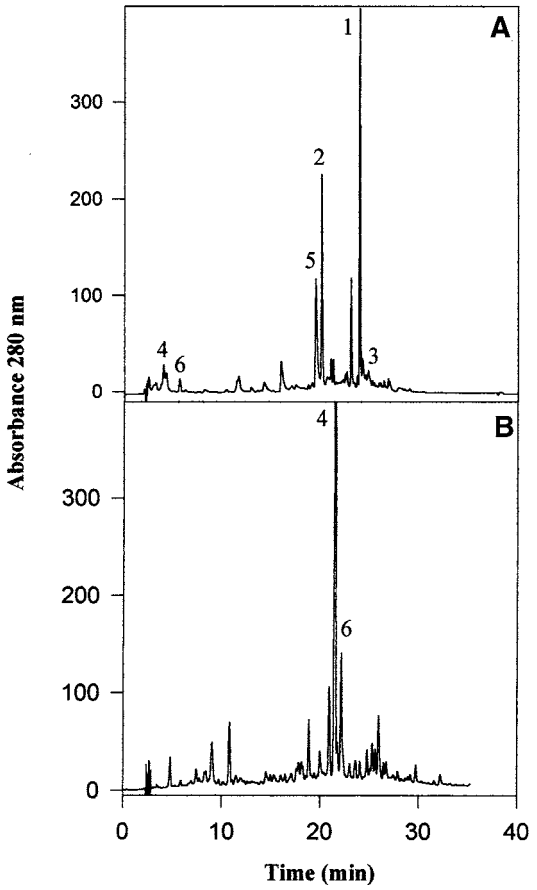

Figure 3. **(A)** Characteristic HPLC chromatogram of a DMSO extract from *R. officinalis* leaves. Peaks: 1, carnosic acid; 2, carnosol; 3, 12-*O*-methylcarnosic acid; 4, rosmarinic acid; 5, genkwanin; 6, isoscutellarein 7-*O*-glucoside. **(B)** Characteristic HPLC chromatogram of an aqueous extract from *R. officinalis* leaves. Peaks: 4, rosmarinic acid; 6, isoscutellarein 7-*O*-glucoside.

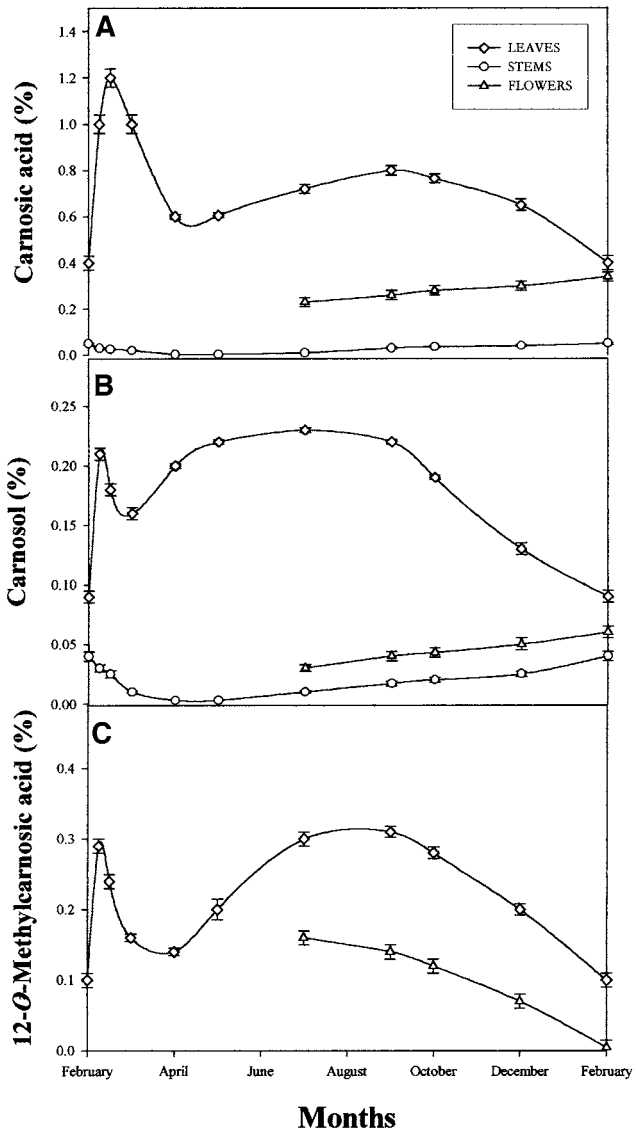

Figure 4. **(A)** Changes in the mean total content (% dry weight) of carnosic acid, according to age, in *R. officinalis* leaves, flowers, and stems. **(B)** Changes in the mean total content (% dry weight) of carnosol according to age in *R. officinalis* leaves, flowers, and stems. **(C)** Changes in the mean total content (% dry weight) of 12-*O*-methylcarnosic acid, according to age, in *R. officinalis* leaves and flowers.

Very low concentrations of carnosic acid and carnosol were observed in roots throughout the growth cycle, and their presence was associated with the youngest steps of this organ. Their concentrations ranged between 0.05 and 0.16% and between 0.01 and 0.08%, respectively, in small roots, but both were absent from roots with a diameter exceeding 0.5 mm.

12-*O*-Methylcarnosic acid was not observed in the vascular system or root. In leaves, the distribution curve of this diterpene was similar to that of carnosic acid during the whole cycle; although, in flowers, the concentration decreased at the same time as in leaves.

Changes in the Levels of Rosmarinic Acid in *Rosmarinus officinalis* Leaves, Flowers, Stems, and Roots during Plant Growth. The concentration of rosmarinic acid (**Figure 5**) reached a maximum during the first stages of leaf growth (5−10 mm), this concentration being higher than those of the diterpenic compounds. Thereafter, rosmarinic acid behaved similarly to carnosic acid and 12-*O*-methylcarnosic acid in leaves. The levels in flowers remained constant, with a small

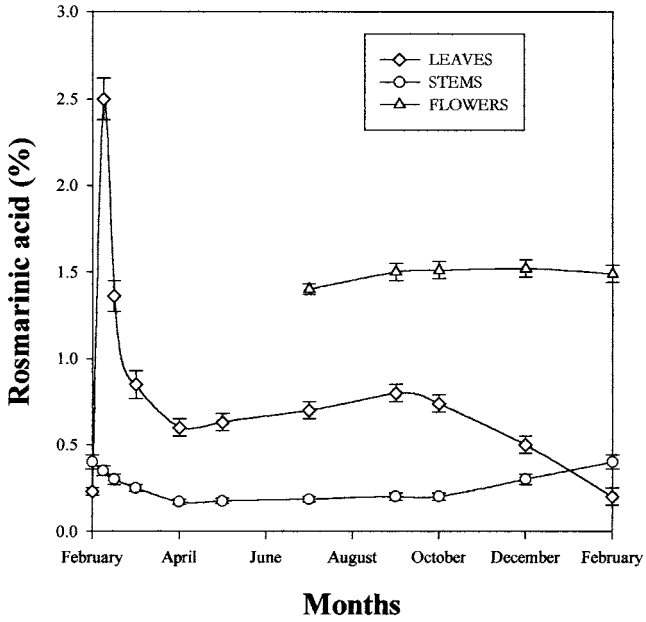

Figure 5. Changes in the mean total content (% dry weight) of rosmarinic acid, according to age, in *R. officinalis* leaves, flowers, and stems.

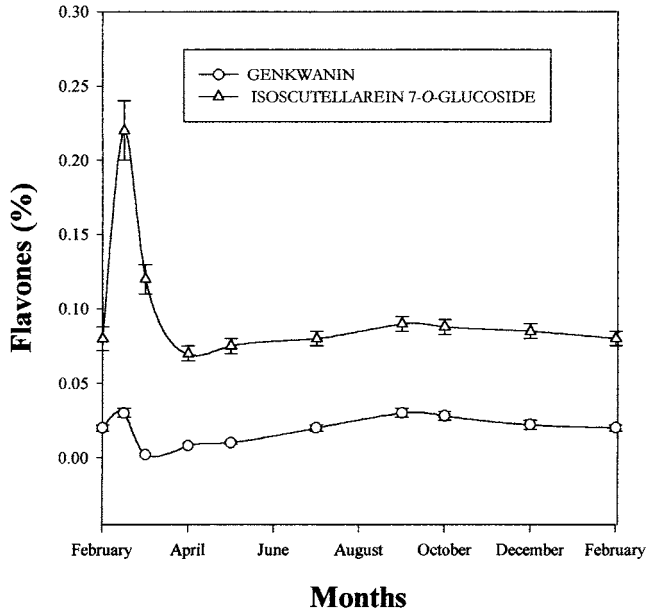

Figure 6. Changes in the mean total content (% dry weight) of genkwanin and isoscutellarein 7-*O*-glucoside, according to age, in *R. officinalis* leaves.

increase during the first months of flowering, and were higher than those in leaves. In stems, the concentration remained constant between April and September, before increasing until it was higher than that in leaves at the end of the cycle. In roots, the concentration was between 0.2% and 0.4%.

Changes in the Levels of Flavones in *Rosmarinus officinalis* Leaves, Flowers, Stems, and Roots during Plant Growth. Genkwanin and isoscutellarein 7-*O*-glucoside (**Figure 6**) reached maximum levels during the first stages of leaf growth (10−15 mm). As was the case with the other polyphenolic compounds, the content decreased until leaf growth ended. Thereafter, the concentrations remained practically constant until the end of the cycle. In flowers, stems, and roots, no flavones were detected.

Quantitative Distribution of Polyphenolic Compounds in Rosemary Leaf Extracts. RO powder and CA 1−CA 5 powders were obtained from *R. officinalis* leaves harvested in

Table 1. Concentration of the Main Polyphenolic Compounds Present in Different Rosemary Leaf Extracts (CA 1–CA 5 and RO Powders) (% Absolute Content)

polyphenolic compounds	CA 1	CA 2	CA 3	CA 4	CA 5	RO
rosmarinic acid	0.35 ± 0.01	1.17 ± 0.02	0.10 ± 0.00	0.07 ± 0.00	0.61 ± 0.01	8.46 ± 0.06
isoscutellarein 7-*O*-glucoside	0.02 ± 0.00	0.89 ± 0.02	0.02 ± 0.00	0.06 ± 0.00	0.01 ± 0.00	4.46 ± 0.04
genkwanin	0.04 ± 0.00	0.12 ± 0.01	0.59 ± 0.01	1.15 ± 0.02	1.50 ± 0.02	0.13 ± 0.01
carnosol	2.88 ± 0.03	7.13 ± 0.06	9.92 ± 0.06	8.00 ± 0.06	13.14 ± 0.09	0.07 ± 0.00
carnosic acid	2.93 ± 0.03	6.13 ± 0.04	9.77 ± 0.07	17.70 ± 0.09	28.30 ± 0.11	0.15 ± 0.01
12-methylcarnosic acid	0.41 ± 0.01	1.00 ± 0.02	2.03 ± 0.02	0.45 ± 0.01	0.59 ± 0.01	0.01 ± 0.00
total	**6.63 ± 0.03**	**16.44 ± 0.06**	**22.43 ± 0.07**	**27.43 ± 0.09**	**44.15 ± 0.11**	**13.28 ± 0.06**

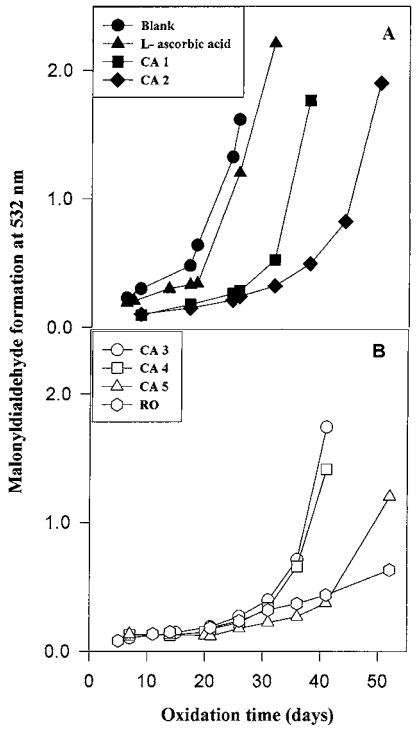

Figure 7. Antioxidant activity in aqueous system. Malonyldialdehyde formation at 532 nm versus time for linoleic aqueous system when L-ascorbic acid, RO powder, and CA 1–CA 5 powders were added in a concentration of 200 ppm.

September 2001 (**Figure 2**). **Table 1** shows the quantitative distribution of the six compounds studied in these extracts. These powders were used in the antioxidant assays.

Antioxidant Activity in Aqueous Systems: TBA Method. The linoleic acid peroxidation was indexed by the measurement of malonyldialdehyde production, using the TBA test (*14*, *22*). **Figure 7** shows the oxidation of linoleic acid versus time as measured by malonyldialdehyde formation at 532 nm, for different linoleic acid solutions when RO and CA1-CA5 powders or L-ascorbic acid were added in a concentration of 200 ppm at 30 °C. In **Figure 7**, malonyldialdehyde formation was delayed with the increase of total polyphenolic compounds in CA powders. The induction time was calculated as the time when the malonyldialdehyde formation is linear. The protection factor was higher for all CA powders (25 to 45 days) than for L-ascorbic acid (17 days) and increased linearly ($r^2 > 0.9695$) with the concentration of polyphenolic compounds contained in these powders (**Table 2**). Despite the low levels of phenolic diterpenes, the RO extract, with its high content of rosmarinic acid, showed the highest antioxidant activity in this aqueous system. The protection factor of RO powder was higher than 55 (**Table 2**).

Table 2. Protection Factor of Rosemary Leaf Extracts (CA 1–CA 5) Compared with Ascorbic Acid (AA) (TBA Method)

antioxidant added, 200 ppm	protection factor[a]	times vs AA
L-ascorbic acid	1.21	1.00
CA 1	1.78	1.47
CA 2	2.21	1.83
CA 3	2.35	1.94
CA 4	2.43	2.01
CA 5	3.31	2.65
RO	>3.92	>3.24

[a] As defined in Materials and Methods.

Table 3. Protection Factor of Rosemary Leaf Extracts (CA 1–CA 5) Compared with α-Tocopherol (E) (Rancimat Method)

antioxidant added, 500 ppm	protection factor[a]	times vs E
α-tocopherol	1.35	1.00
CA 1	1.94	1.43
CA 2	2.36	1.75
CA 3	2.80	2.07
CA 4	2.78	2.06
CA 5	3.65	2.70

[a] As defined in Materials and Methods.

Antioxidant Activity in Lipid Systems: Rancimat Method. Table 3 shows the protection factor when CA powders were added in a concentration of 500 ppm to lard at 120 °C. Lard without additives has, by definition, a protection factor of 1, which increased to 1.35 when 500 ppm of α-tocopherol was added. The addition of CA powders provided protection factors ranging from 1.94 to 3.65, with a linear correlation ($r^2 = 0.986$). RO powder was not used in these experiments, because it is insoluble in lipid systems. The CA powder with the lowest diterpene phenol content (6.63%) had a protection factor in lard 1.4 times higher than α-tocopherol at the same concentration, wich increased to 2.7 times for CA powders containing 44.2% phenolic compounds (**Table 3**).

DISCUSSION

Distribution and Biosynthesis of Polyphenolic Compounds. Three different polyphenol skeletons were present in *R. officinalis* (phenolic diterpenes, caffeoyl derivatives, and flavones), each showing a characteristic behavior and distribution during the vegetative cycle. Only in leaves were all three groups present, and in each case, the highest accumulation rate was related with the young stages of development, a behavior similar to that observed in other plants (*23*). The levels of these polyphenolic compounds decreased rapidly with age, due to their dilution during leaf growth, but different profiles were observed for each group of polyphenols.

Previous studies reported the influence of leaf age and demostrated a strong seasonal variation in the concentration of

carnosic acid and other phenolic diterpenes (*16*, *17*). In general, there is a well-established positive relationship between the intensity of solar radiation and phenol formation in plants, although the concentration of carnosic acid in rosemary leaf decreases with increasing solar radiation and temperature (*8*, *17*, *18*, *24*). Our results, described in **Figure 4**, confirm and complete these previous findings for phenolic diterpenes, with the high concentration of these polyphenols during early leaf growth stages being related to the intense cellular division that occurs at this time. However, the distribution of these phenolic diterpenes, particularly of carnosic acid, suggests the occurrence of a degradative process involving oxidative and/or enzymatic conversions to form lactones such as rosmanol and isorosmanol, and also the methylation of their structures (*2*, *5*, *8*, *9*, *17*, *18*, *24*). Carnosol shows a different distribution from that of carnosic acid in the March−July period, which is probably related to the partial biotransformation of carnosic acid in to carnosol (*2*, *8*, *9*, *24*).

As has been described in other plants (*25*), the presence in stems of some of the polyphenols studied in this investigation suggests a close relationship between the different processes of biosynthesis, degradation, and transport involved in the distribution of these polyphenols in the plant organs. Both carnosic acid and carnosol were present in stems during the young stages of leaves, which suggests a transport phenomenon toward younger leaves. When leaf growth finished, both these phenolic diterpenes disappeared from the vascular system. A second increase in the levels of carnosic acid and carnosol was observed in stems and flowers, simultaneously, as they decreased in leaves. At the end of the cycle studied, the content of phenolic diterpenes, especially carnosic acid, was similar in leaves and flowers. This behavior suggests the transport of phenolic diterpenes from leaves to flowers (new organ) through the vascular system. However, the initial high levels of these compounds in flowers during flowering suggest an in situ biosynthetic mechanism. The distribution of these phenolic diterpenes in this organ during its development mainly depends on two phenomena: endogenous biosynthesis and transport from leaves. The fact that 12-*O*-methylcarnosic acid was absent from stems suggests that the presence of this acid in flowers is due to endogenous biosynthesis alone. Its concentration decreased during the flowering cycle due to the above-mentioned degradative/oxidative processes.

Of all the polyphenols, rosmarinic acid showed the highest concentration in all the organs. Its distribution curve (**Figure 5**) showed a maximum during the first stages of leaf growth but decreased sharply when the leaves reached 10−15 mm in length, probably due to the dilution process involved in leaf growth and to its transport to younger leaves. Hence, this polyphenol is present in the vascular system throughout the cycle studied. In addition, degradative process of this compound in the plant were not described. Similarly to phenolic diterpenes, the levels of this compound in stems increased after the summer, as it decreased in leaves. The flower showed the highest levels of rosmarinic acid, and in this organ, the concentration was practically constant. Its presence in flowers would result from a combination of endogenous biosynthesis and transport from leaves through the stems. The distribution of this acid in all the organs suggests that, in the first stages of flower growth, its levels were due to its in situ biosynthesis and that, in the last stages, were increased by transport phenomena.

Similarly to the other polyphenols, the flavones, isoscutellarein 7-*O*-glucoside and genkwanin (**Figure 6**), showed maximum levels in the young stages of leaf growth. The subsequent decrease in concentration was due to the above-mentioned dilution process alone, because the total amount per leaf remained constant (data not shown). Their distribution suggests that only leaves are able to biosynthesize these flavonoids, which were lacking in flowers and stems, and that there is no transport process from leaves. The absence of flavones in the vascular system confirms previous findings in other plant materials (*25*). In roots, only small amounts of rosmarinic acid, carnosic acid, and carnosol were detected.

Antioxidant Activity. The polyunsaturated fatty acids present in cell membranes are easily oxidized both by enzymatic and autoxidative peroxidation via free radical chain reactions. Lipid peroxidation can be induced by free radicals (superoxide and hydroxyl) and by the singlet oxygen produced in biological systems. Thus, lipid peroxidation may be prevented by free radicals scavengers or by singlet oxygen quenchers. In this study, the results obtained concerning antioxidant activity of the different extracts of *R. officinalis* in aqueous and lipid systems were similar to those reported by other authors (*2*−*5*, *7*, *10*, *12*, *14*, *15*, *22*, *26*).

It has been shown that the addition of polyphenols reduces the level of oxidation compounds during the autoxidation of the polyunsaturated fatty acid (linoleic acid). It should be noted that RO powder, with its high concentrations of rosmarinic acid and the flavone isoscutellarein 7-*O*-glucoside, showed the highest antioxidant activity of all rosemary compounds in this aqueous medium. On the other hand, an increase in phenolic diterpenes, the compounds mainly responsible for the antioxidant activity of CA extracts, led to the prolongation of linoleic stability in an ethanol−water system. As was observed in aqueous systems for the autoxidation of linoleic acid, concentrations of phenolic diterpenes in CA powders led to longer induction periods during lard oxidation.

The linear correlation coefficients ($r^2 > 0.96$) between the protection factors and the content of phenolic diterpenes in CA powders, in both aqueous and lipids systems, clearly indicates that these compounds, especially carnosic acid and carnosol, are responsible for the high antioxidant activity of rosemary extracts, as was reported previously (*2*−*5*, *12*, *14*, *15*, *26*, *27*). Rosemary extracts are therefore excellent antioxidants in both aqueous (particularly RO powder) and lipid systems (CA powder).

The antioxidant activity mechanism of rosemary extracts is similar to that of other polyphenols and flavonoids (*18*, *28*). The presence of a catechol group in the aromatic ring (C_{11}−C_{12}) of the rosemary phenolic diterpene skeleton is probably the most important structural element in the antioxidant activity of these compounds. In rosmarinic acid, the presence of two catechol structures conjugated with a carboxylic acid group increases the antioxidant activity in aqueous or aqueous−alcoholic media.

The antioxidant activity of the CA extracts containing the lowest levels of phenolic diterpenes is higher than the antioxidant normally used as reference in aqueous (L-ascorbic acid) and lipid (α-tocopherol) systems. The presence of flavones in the rosemary extract suggests a possible synergistic behavior in its radical scavenging capacity.

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