

Communication

Antioxidant Activity of a New Aromatic Geranyl Derivative of the Resinous Exudates from *Heliotropium glutinosum* Phil.

Brenda Modak ^{1,*}, Macarena Rojas ¹, René Torres ¹, Jesús Rodilla ² and Federico Luebert ³

¹ Universidad de Santiago de Chile. Facultad de Química y Biología. Departamento de Ciencias del Ambiente. Casilla 40, correo 33, Santiago, Chile, Tel. (+56)-02-6812575; Fax. (+56)-02-6812108; E-mails: maquitarojas@yahoo.es; rtorres@usach.cl

² Departamento de Química, Universidade da Beira Interior, U. I&D Materiais Textêis e do Papel. 6201-001Covilhã, Portugal; E-mail: rodilla@ubi.pt

³ Universidad de Chile. Facultad de Ciencias Forestales. Departamento de Silvicultura. Casilla 9206, Santiago, Chile; E-mail: fluebert@uchile.cl

* Author to whom correspondence should be addressed; e-mail: bmodak@lauca.usach.cl

Received: 5 April 2007; in revised form: 15 May 2007 / Accepted: 15 May 2007 / Published: 21 May 2007

Abstract: *Heliotropium glutinosum* Phil. (*Heliotropiceae*) is a resinous bush that grows at a height of 2000 m in Chañaral, Chile. From the resinous exudates of *Heliotropium glutinosum* Phil. a new aromatic geranyl derivative: 4-methoxy-3-[(2)-7'-methyl-3'-hydroxymethyl-2',6'-octadienyl] phenol (**1**) and three flavonoids: 5,3'-dihydroxy-7,4'-dimethoxyflavanone (**2**), 5,4'-dihydroxy-7-methoxyflavanone (**3**) and 4'-acetyl-5-hydroxy-7-methoxyflavanone (**4**) were isolated and their structures were determined. Their antioxidant activity were evaluated using the bleaching of ABTS and DPPH derived cation radical methods and expressed in terms of FRE (fast reacting equivalents) and TRE (total reacting equivalents), where FRE is a good measure of the quick protection of a given compound against oxidants and TRE measures the degree of long-term protection of the antioxidant, or how effective it is against a strong oxidative stress.

Keywords: *Heliotropium glutinosum*; geranyl derivatives; flavonoids; antioxidant capacity; resinous exudates.

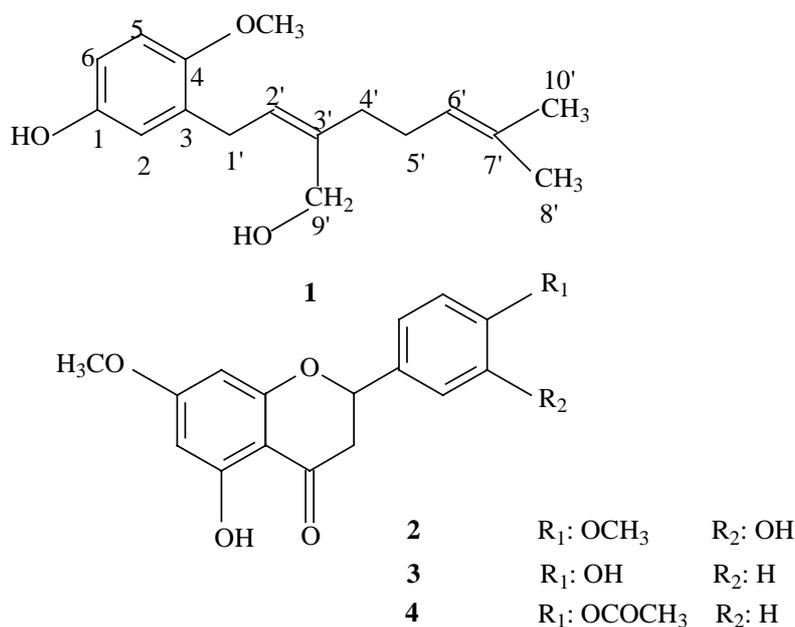
Introduction

Flavonoids are a large group of naturally phenolic compounds almost ubiquitous in higher plants. Many studies have suggested that flavonoids exhibit chemical properties that translate into biological activities. One of the most interesting is their antioxidant activity, which is due to their ability to reduce free radical formation and also to scavenge free radicals. On the other hand, in our laboratory we have shown that the resinous exudates isolated from species of *Heliotropium* genus are characterized by the presence of compounds with antioxidant properties as part of a protective role against the oxidative stress imposed on the species [1]. In a continuation of our research on the chemical composition of the resinous exudates of Chilean desert plants of the genus *Heliotropium* [1-10] we have analyzed the secondary metabolites present in the resin produced by *Heliotropium glutinosum* Phil. This specie is a resinous bush that grows in Chañaral (III region, Chile) at a height of 2000 m. Like other members of *Heliotropium* genus, the resin of this species was characterized by the presence of flavonoids, but the protection model was complemented with a geranyl phenol. The antioxidant activity of the isolated phenols was measured in terms of their rate of radical bleaching with cations radicals derived from ABTS [2,2'-azinobis(3-ethylbenzoline-6-sulfonic acid)] and DPPH [(1,1-diphenyl-2-picrylhydrazyl)] and of the stoichiometry in microequivalents of Trolox[®] by the determination of its fast (FRE) and total (TRE) reacting equivalents [1].

Results and Discussion

The secondary metabolites present in the resin produced by *Heliotropium glutinosum* Phil. are a new phenolic geranyl derivative, namely 4-methoxy-3-[(2)-7'-methyl-3'-hydroxymethyl-2',6'-octadienyl]phenol (**1**) and three flavonoids: 5,3'-dihydroxy-7,4'-dimethoxyflavanone (**2**), 5,4'-dihydroxy-7-methoxyflavanone (**3**) and 4'-acetyl-5-hydroxy-7-methoxyflavanone (**4**) (Figure 1).

Figure 1. Compounds isolated from *Heliotropium glutinosum* Phil.



The flavonoids **2-4** have all been previously isolated by us from *H. chenopodiaeum* var. *ericoideum*, *H. stenophyllum* and *H. chenopodiaeum* var. *chenopodiaeum* and identified [2,3,6]. Their structures were confirmed by comparison to literature data. The new compound was characterized by its spectroscopic data as 4-methoxy-3-[(2)-7'-methyl-3'-hydroxymethyl-2',6'-octadienyl]phenol (**1**). The presence of hydroxyl groups was evident from the IR absorption band at $3,402\text{ cm}^{-1}$. The $^1\text{H-NMR}$ spectrum of **1** showed signals at δ 1.58 ppm (s, 3H, H-10') and δ 1.67 ppm (s, 3H, H-8'), which could be attributed to two methyl groups located on a unsaturated system, as indicated by their relation with the carbons 6' and 7'. The protons at δ 2.15 ppm (s, 4H, H-4' and H-5') were related with the $^{13}\text{C-NMR}$ signals at δ 35.6 ppm (C-4') and 26.9 ppm (C-5') in the HMQC experiment. A doublet at δ 3.37 ppm ($J=7.8\text{ Hz}$, 2H, H-1'), coupled to H-2' (5.41 ppm) was shown by a COSY experiment, to correspond to a benzylic methylene connected with the following carbons: 2, 3, 4, 2' and 3' (HMBC). A signal at δ 3.78 ppm (s, 3H, OCH₃) could be attributed to methoxyl group. These protons were related with the $^{13}\text{C-NMR}$ signal at δ 56.1 ppm and were shown to be coupled to C-4 (151.2 ppm) in the HMBC experiment. The signal at δ 4.21 ppm (s, 2H, H-9') was attributed to two protons linked to an OH group, that correlated with the $^{13}\text{C-NMR}$ signal at δ 60.4 ppm (C-9') in the HMQC experiment and was shown to be connected with δ 126.5 (C-2'), δ 139.2 (C-3') and δ 35.60 (C-4') in the HMBC experiment. At δ 5.10 and δ 5.41 ppm two signals (t, 1H, $J = 6.0\text{ Hz}$, H-6' and t, 1H, $J = 8.0\text{ Hz}$, H-2', respectively) were observed which were correlated with the $^{13}\text{C-NMR}$ signals at δ 124.0 ppm (C-6') and 126.5 (C-2'). The signals at δ 5.10 and δ 5.41 ppm were shown to be coupled to H-5' and H-1', respectively, by a COSY experiment. The $^1\text{H-NMR}$ spectrum also showed three aromatic protons at δ 6.72 (d, 1H, $J = 8.4\text{ Hz}$, H-5), this signal was related with the carbon signal at δ 111.8 ppm (C-5); at δ 6.66 (s, 1H, H-2) correlated by the HMQC experiment with the carbon signal at δ 116.9 ppm (C-2) and δ 6.64 (d, 1H, $J = 8.4\text{ Hz}$, H-6), which showed a correlation with the carbon signal at δ 113.3 ppm (C-6). Finally, the NOESY experiment showed coupling between H-5' with the CH₂OH and H-5' with the CH₃ at δ 1.67 ppm. These results confirmed the *cis* position for the 8' methyl group. The assigned structure was confirmed by the mass spectra, with a M+H ion at m/z 277.1779, consistent with the formula C₁₇H₂₄O₃. This is the first report of compound (**1**).

Antioxidant activity

The antioxidant activity of the compounds **1-4** and of the resin were evaluated using bleaching of the ABTS and DPPH derived cation radical methods and expressed in terms of FRE (fast reacting equivalents) and TRE (total reacting equivalents). FRE is a measure of the quick protection of a given compound against oxidants and TRE measures the degree of long-term protection of the antioxidant [1,11]. In general, the results with ABTS showed a good relation between the number of phenolic groups presents in the compounds and the FRE or TRE indexes.

The value of the FRE index obtained for **1** with ABTS showed that the sole phenolic group reacts quickly and the high TRE index is attributed to the methoxyl group in the *para* position. This substitution increases the production of a stable radical by an inductive effect, improving the antioxidant capacity [12]. The activity of compound **1** turned out be similar to that of other substituted monophenols [13].

For the flavonoid **2** the TRE index showed the presence of two phenolic groups, but the FRE index indicates that only one OH acts quickly, while the flavonoid **3** showed a very low antioxidant activity, owing to the fact that its phenolic hydroxyl acts slowly. On the other hand, the flavonoid **4** showed that its two phenolic groups acts quickly with ABTS, however, the high value of the TRE index is not interpretable. The results with DPPH are smaller compared with ABTS, but they follow a similar tendency, mainly with regards to the TRE index (Table 1). These values are within the range obtained from other flavonoids [9]. On the other hand, it is possible to affirm that these results are markedly depends upon the stable free radical employed, because the kinetics of the process are usually complex [14].

Table 1. Stoichiometric coefficients (number of free radicals consumed per molecule of additive) for the reaction of the radicals derived from ABTS and DPPH with the isolated compounds **1-4** at 15 s. (FRE) and 15 min. (TRE).

Compounds	Number of phenolic groups	ABTS		DPPH	
		FRE	TRE	FRE	TRE
1	1	1.6	2.4	0.003	0.057
2	2	1.5	2.7	0.007	0.013
3	1	0.02	1.3	0.005	0.006
4	2	2.3	11.8	0.010	0.057
Trolox[®]	1	1.0	1.0	1.0	1.0

The levels of antioxidant activity evaluated for the total extract of *Heliotropium glutinosum* Phil. is high and similar to that of resinous exudates isolated from other *Heliotropium* species (Table 2) [1,9]. The antioxidant potential of this resin may be due to the presence of the phenols isolated from it. These phenols showed a good activity, mainly in terms of the long protection. Therefore, these compounds must play a protective role against the oxidative stress imposed by the environmental conditions in which they are developed.

Table 2. Concentration of the antioxidants present in the resinous exudates from *Heliotropium* species.

Concentration of antioxidants present in the extracts.	ABTS	DPPH
Resin of <i>H. glutinosum</i>	1.9	0.14
Resin of <i>H. huascoense</i>	2.0	0.058
Resin of <i>H. filifolium</i>	2.4	0.28

The concentrations in the resinous exudates were obtained by monitoring their capacity to bleach ABTS and DPPH radicals. The equivalent antioxidant potential is informed in terms of TROLOX[®] equivalents.

Conclusions

From the extract resinous of *Heliotropium glutinosum* Phil. a new phenolic geranyl derivative identified as 4-methoxy-3-[(2)-7'-methyl-3'-hydroxymethyl-2',6'-octadienyl] phenol (**1**) and three flavonoids, isolated previously from other species of the genus *Heliotropium*: 5,3'-dihydroxy-7,4'-dimethoxyflavanone (**2**), 5,4'-dihydroxy-7-methoxyflavanone (**3**) and 4'-acetyl-5-hydroxy-7-methoxyflavanone (**4**) were isolated and the antioxidant activity of these compounds was evaluated.

Other species of the genus *Heliotropium* have been shown to produce resinous exudates with high concentrations of phenolic compounds, mainly flavonoids, in order to prevent the oxidative degradation of the resin that protects the plant [1-9]. In *H. glutinosum* the antioxidant activity was due not only to flavonoids, but also to compound **1**. The kinetic values showed a similar mechanism for the two types of phenolic compounds. Studies of the relationships between concentrations of compounds through the year are currently under way.

Experimental Section.

General

¹H- (400 MHz) and ¹³C-NMR spectra (100 MHz) were recorded in CDCl₃ on a Bruker Avance DRX400 spectrometer with TMS as internal standard. IR spectra were recorded on a Perkin-Elmer 735-B spectrophotometer. Mass spectra were obtained with a Fisons Autospec-Q VG-Analytical instrument. The optical rotations were measured with a Perkin-Elmer 241 polarimeter. The melting points were measured on a Kofler micro melting instrument and are not corrected. Known compounds were identified by comparison of their spectroscopic data with those in the literature and by co-chromatography with authentic samples. Silica gel 60 (70-230 mesh ASTM; 63-200µm) for open column chromatography (CC) and GF₂₅₄ for analytical TLC were purchased from Merck Ltd. (Germany). DPPH and ABTS were purchased from Sigma-Aldrich Chemical Co. (USA). All solvents and chemicals used were of analytical grade.

Plant material

Heliotropium glutinosum Phil. was collected in January 2004 from Quebrada de Potrerillos, Province of Chañaral, 3rd Region of Atacama, Chile (26° 24` S, 69° 32` W). A voucher specimen (HG 2007) was deposited at the Herbarium of Natural History Museum, Santiago of Chile.

Extraction and isolation

Resinous exudates were extracted by immersing the fresh plant for between 15 and 30 s. in dichloromethane [6]. The extract of the resinous exudates obtained from *Heliotropium glutinosum* Phil. was subjected to successive column and preparative chromatography that led to isolation of the various compounds. The fresh plant of *H. glutinosum* was dipped into dichloromethane for 30 s. The extract was concentrated to give a resin residue (132 g). The resin (15 g) was separated into nine

fractions by CC (silica gel, mixtures of increasing polarity of dichloromethane-methanol as eluents). Fraction 5 (1.3 g) was purified by PTLC on silica gel, eluting with hexane-ethyl acetate (60:40) to give 139.0 mg of 4-methoxy-3-[(2)-7'-methyl-3'-hydroxymethyl-2',6'-octadienyl]phenol (**1**): $[\alpha]_D^{24} = -1^\circ$ (c 1.9, CH₃OH); IR (KBr) ν_{\max} 3402, 2922, 2852, 1720, 1599 cm⁻¹; ¹H-NMR: δ 1.58 (s, 3H, H-10'), 1.67 (s, 3H, H-8'), 2.15 (s, 4H, H-4' and H-5'), 3.37 (d, $J=7.8$, 2H, H-1'), 3.78 (s, 3H, OCH₃), 4.21 (s, 2H, H-9'), 5.10 (t, 1H, H-6'), 5.41 (t, 1H, H-2'), 6.66 (s, 1H, H-2), 6.64 (d, $J=8.4$, 1H, H-6), 6.72 (d, $J=8.4$, 1H, H-5); ¹³C-NMR: δ 17.7 (C-10'), 25.6 (C-8'), 28.7 (C-1'), 26.9 (C-5'), 35.6 (C-4'), 56.1 (OCH₃), 60.4 (C-9'), 111.8 (C-5), 113.0 (C-6), 116.9 (C-2), 124.0 (C-6'), 126.5 (C-2'), 130.4 (C-3), 131.7 (C-7'), 139.2 (C-3'), 149.6 (C-1), 151.2 (C-4); HREIMS: M+H ion m/z 277.1779 (calcd. for C₁₇H₂₄O₃: 276.3748).

Antioxidant capacity: Antioxidant activities determined employing the ABTS derived radical cation. The procedures employed were similar to those described in [11]. ABTS derived radical cation was prepared by treating ABTS (65 μ M) with MnO₂ (25 mg/mL) in phosphate buffer solution (10 μ M, pH 7, 5 mL). The solution was centrifuged and filtered. The filtrate showed the typical green-blue colour of the radical solution with absorbance 734 nm (ϵ 0.0039). Aliquots of 5 μ L of the ethanolic solution containing the antioxidant were added to 3 mL of the radical solution. The decrease in the absorbance at 734 nm, due to the consumption of preformed radical, was followed as a function of the elapsed time.

Antioxidant activities determined employing the DPPH radical. Solutions of antioxidant were prepared in ethanol (c 1 mg/mL). Aliquots of these solutions (5 μ L) were added to the ethanolic radical solution (3 mL, 1.29 μ M). Changes in the absorbance of the solution elicited by addition of the solutions containing the antioxidants, were measured at 517 nm as a function of the elapsed time. The antioxidant potential in the resinous exudates was obtained by monitoring their capacity to bleach ABTS and DPPH radicals. The equivalent antioxidant potential was obtained, in terms of Trolox[®] equivalents, with the formula: $X = f \Delta A_{\text{sample}} / \Delta A_{\text{Trolox}}^{\text{®}}$, where f is a dilution factor equal to the ratio between the volume of free radical solution in the reaction cell and the exudates aliquot; ΔA_{sample} is the decrease in ABTS or DPPH absorbance produced by the sample aliquot incorporation, and ΔA_{Trolox} is the decrease in absorbance elicited by 1 mM Trolox[®] concentration.

Acknowledgements

This work was supported by FONDECYT 1030813, 1070121 and DICYT 20541MC. Also we are thankful to the General NMR Service of the University of Salamanca, Spain.

References

1. Lissi, E.; Modak, B.; Torres, R.; Escobar, J.; Urzúa, A. Total antioxidant potential of resinous exudates from *Heliotropium* species and a comparison of the ABTS and DPPH methods. *Free Rad. Res.* **1999**, *30*, 471- 477.

2. Villarroel, L.; Torres, R.; Urzúa, A. Compuestos fenólicos en el exudado resinoso de *Heliotropium stenophyllum*. Determinación estructural y efectos antialimentario y antioxidantes. *Bol. Soc. Chil. Quím.* **1991**, *36*, 169-174.
3. Urzúa, A.; Villarroel, L.; Torres, R.; Teillier, S. Flavonoids in the resinous exudate of Chilean *Heliotropium* species from *Cochranea* Section. *Biochem. Syst. Ecol.* **1993**, *21*, 744.
4. Torres, R.; Urzúa, A.; Villarroel, L.; Delle Monache, F.; Gacs-Baitz, E. Filifolinol, a rearranged geranyl aromatic derivative from the resinous exudates of *Heliotropium filifolium*. *Phytochemistry*. **1994**, *36*, 249-250.
5. Torres, R.; Modak, B.; Villarroel, L.; Urzúa, A.; Delle Monache, F.; Sanchez-Ferrando, F. Flavonoides del exudado resinoso de *Heliotropium sinuatum*. *Bol. Soc. Chil. Quím.* **1996**, *41*, 195-197.
6. Urzúa, A.; Modak, B.; Villarroel, L.; Torres, R.; Andrade, L. Comparative flavonoid composition of the resinous exudates from *Heliotropium chenopodiaceum* var. *chenopodiaceum* and *H. chenopodiaceum* var. *ericoideum*. *Biochem. Syst. Ecol.* **1998**, *26*, 127-130.
7. Urzúa, A.; Modak, B.; Villarroel, L.; Torres, R.; Andrade, L.; Mendoza, L.; Wilkens, M. External flavonoids from *Heliotropium megalanthum* and *H. huascoense* (Boraginaceae). *Bol. Soc. Chil. Quím.* **2000**, *45*, 23-29.
8. Urzúa, A.; Modak, B.; Torres, R. Identification of a new aromatic geranyl derivative in the resinous exudate of *Heliotropium filifolium* (Boraginaceae). *Bol. Soc. Chil. Quím.* **2001**, *46*, 175-178.
9. Modak, B.; Torres, R.; Lissi, E.; Delle Monache, F. Antioxidant capacity of flavonoids and a new arylphenol of the resinous exudates from *Heliotropium sinuatum*. *Nat. Prod. Res.* **2003**, *17*, 403-407.
10. Modak, B.; Contreras, L.; González-Nilo, D.; Torres, R. Structure-Antioxidant Activity relationships of flavonoids isolated of resinous exudates from *Heliotropium sinuatum*. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 309-312.
11. Aliaga, C.; Lissi, E. Reactions of the radical cation derived from 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS^{•+}) with amino acids. Kinetics and mechanism. *Can. J. Chem.* **2000**, *78*, 1052-1059.
12. Leu, T. The molecular mechanism for the antitumorigenic effect of curcumin. *Curr. Med. Chem. Anti-Cancer Agents.* **2000**, *2*, 357-370.
13. Campos, A.; Lissi, E. Evaluation of the antioxidant capacity of herbal teas by a procedure based on the bleaching of ABTS radical cations. *Bol. Soc. Chil. Quím.* **1995**, *40*, 375-381.
14. Perez, D.; Leighton, F.; Aspee, A.; Aliaga, C.; Lissi, E. A comparison of methods employed to evaluate antioxidant capabilities. *Biol. Res.* **2000**, *33*, 71-77.

Sample Availability: Samples of the compound **1** are available from authors.