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## Presence of 3-(pentadec-10-enyl)-catechol allergen in the epicuticular components of *Lithrea caustica* (Anacardiaceae)

[Presencia del alérgeno 3-(pentadec-10-enil)-catecol en los componentes epicuticulares de *Lithrea caustica* (Anacardiaceae).]

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### Abstract

Epicuticular components were obtained using methylene chloride extraction of fresh leaves from two populations of *Lithrea caustica*. The methylene chloride extracts were analyzed using GC and GC-MS. The extracts from both sampled populations showed a mixture of a hydrocarbon fraction of n-alkanes from C-21 to C-33 as their main components and small amounts of monoterpene hydrocarbons. The allergen 3-(pentadec-10-enyl)-catechol was also identified in the epicuticular sample in very different proportions in both extracts. A second extract obtained after the epicuticle had been removed from the sample revealed oxygenated monoterpenes, sesquiterpene hydrocarbons and an increased amount of the allergen 3-(Pentadec-10-enyl)-catechol. These results demonstrate that the cuticle hydrocarbons of the leaves function as a lipophylic barrier that controls allergen release.

**Keywords:** *Lithrea caustica*; Anacardiaceae; epicuticular components; hydrocarbons; terpenoids; 3-(Pentadec-10-enyl)-catechol

### Resumen

Los componentes epicuticulares se obtuvieron mediante la extracción con cloruro de metileno de hojas frescas de dos poblaciones de *Lithrea caustica*. Los extractos de cloruro de metileno fueron analizados mediante CG y CG-EM. Los extractos de ambas poblaciones mostraron una mezcla de una fracción de hidrocarburos n-alcános de C-21 a C-33 como sus componentes principales y pequeñas cantidades de hidrocarburos monoterpenicos. El alérgeno 3 - (pentadec-10-enil)-catecol también fue identificado en epicutícula en proporciones muy diferentes en ambos extractos. Un segundo extracto obtenido después que la epicutícula había sido eliminada de la muestra mostró monoterpenos oxigenados, hidrocarburos sesquiterpenos y una mayor cantidad del alérgeno 3 - (Pentadec-10-enil)-catecol. Estos resultados demuestran que los hidrocarburos de la cutícula de las hojas funcionan como una barrera lipofílica que controla la liberación del alérgeno.

**Palabras Clave:** *Lithrea caustica*; Anacardiaceae; componentes epicuticulares; hidrocarburos; terpenoides; 3 - (pentadec-10-enil)-catecol.

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## INTRODUCTION

"Litre" (*Lithrea caustica* (Mol) Hooker & Arnott), is a common evergreen tree, shrub or creeping habit plant, from 0.5 to 3 m high, endemic to Chile. Is distributed among Regions IV & IX (Riedeman *et al.*, 2001; Rodríguez *et al.*, 1983), especially along the coast, coastal mountains and the foothills of the Andes.

The species is known to produce contact dermatitis (Gambaro *et al.*, 1986; Muñoz *et al.*, 1981), causing severe reactions in some people. The fruits are edible and used to make alcoholic beverages; a tincture of the leaves is applied in homeopathic doses to treat scaly skin diseases (Muñoz *et al.*, 1981).

In a previous study, 3-(Pentadec-10-enyl)-catechol (**1**) was identified as the compound responsible for the Litre's allergenic properties (Gambaro *et al.*, 1986). In an attempt to show that the allergenic compounds were volatilized, especially in times of heat, seven monoterpene hydrocarbons were identified by Micro Solid Phase Extraction (SPME) (Garbarino *et al.*, 2002), however none of the compounds had allergenic properties. Furthermore, as the plant material analyzed was not fresh the results can hardly be correlated with what happens in nature.

Rural myths indicate that certain types of Litre are more "aggressive" and cause more intense dermatitis. This may be related to differences in abiotic factors, particularly water availability and in special high temperatures (Muñoz *et al.*, 1981). The "aggressive Litre" is found in dry areas in the summer month while less aggressive plants can be found at the same time of year in different environments.

Abiotic factors determine the thickness and chemical composition of epicuticular waxes that cover the leaves, which act as a lipophylic barrier to the spread of allergen compounds from the esquizogenic vessels where they accumulate (Montenegro *et al.*, 1984).

This study reports, for the first time, a comparison of the chemical composition of the leaf

surface of Litre collected in two areas. Within its wide distribution the following sample areas were chosen: 1) Metropolitan Region, Lo Prado Pass (33° 10' S, 70° W), 2) Metropolitan Region, Farellones road (31° 21.5' S, 70° 21' S).

## RESULTS AND DISCUSSION

The epicuticular components of the extracts (%) are listed in Table 1.

**Table 1**  
**Epicuticular components (%)**

FRE-1	0.428
LPPE	0.257
FRE-2	0.125

FRE-1: First Farellones Road Extract;

LPPE: Lo Prado Pass Extract;

FRE-2: Second Farellones Road Extract;

The composition of the epicuticular components from leaf extracts of *Lithrea caustica* (%), are listed in Table 2. In the methylene chloride extract of FRE-1, 84.5% of the compounds were identified. The extract shows as main components a hydrocarbon fraction of n-alkanes from C21 to C33 that corresponds to 76.5% of the identified compounds. Additionally, small amounts of 6 hydrocarbon monoterpenes (2.6%) and 4.8% of the allergenic compound 3-(Pentadec-10-enyl)-catechol (**1**) were identified.

In the methylene chloride extract of LPPE, 85.9% of the compounds were identified. The extract shows a hydrocarbon fraction of n-alkanes from C21 to C33 that correspond to 64.5% of the identified compounds and are the main component. Additionally, small amounts of 6 hydrocarbon monoterpenes (2.9%) and 18.5% of the allergen 3-(Pentadec-10-enyl)-catechol (**1**) were identified.

**Table 2**  
Composition (%) of the epicuticular components of leaves extracts of *Lithrea caustica*

Compounds	RI	FRE-1	LPPE	FRE-2
<b>1 (Z)-3-hexen-1-ol</b>	861	-	-	2.1
<b>2 α-Pinene</b>	934	0.5	0.6	-
<b>3 β-Pinene</b>	976	0.1	0.2	-
<b>4 Myrcene</b>	992	1.1	1.2	-
<b>5 α-Phellandrene</b>	1004	0.1	0.1	-
<b>6 p-Cymene</b>	1024	0.4	0.3	-
<b>7 Limonene</b>	1032	0.4	0.5	-
<b>8 Isopulegol</b>	1160	-	-	1.3
<b>9 Menthol</b>	1173	-	-	1.4
<b>10 α-Guaiene</b>	1448	-	-	6.0
<b>11 Caryophyllene</b>	1452	-	-	1.0
<b>12 3,11-Eudesmadien</b>	1490	-	-	6.5
<b>13 Phytone</b>	1854	0.6	-	tr
<b>14 C<sub>21</sub> H<sub>44</sub></b>	2100	0.3	-	-
<b>15 C<sub>22</sub> H<sub>46</sub></b>	2200	0.4	-	-
<b>16 C<sub>23</sub> H<sub>48</sub></b>	2300	1.3	-	tr
<b>17 C<sub>24</sub> H<sub>50</sub></b>	2400	1.4	-	tr
<b>18 C<sub>25</sub> H<sub>52</sub></b>	2500	4.9	2.6	1.2
<b>19 C<sub>26</sub> H<sub>54</sub></b>	2600	2.2	0.2	tr
<b>20 3-(Pentadec-10-enyl)-catechol</b>	2671	4.8	18.5	67.5
<b>21 C<sub>27</sub> H<sub>56</sub></b>	2700	12.3	14.3	1.4
<b>22 C<sub>28</sub> H<sub>58</sub></b>	2800	2.1	1.6	tr
<b>23 C<sub>29</sub> H<sub>60</sub></b>	2900	13.7	26.5	0.7
<b>24 C<sub>30</sub> H<sub>62</sub></b>	3000	1.1	-	-
<b>25 C<sub>31</sub> H<sub>64</sub></b>	3100	34.3	17.4	1.8
<b>26 C<sub>32</sub> H<sub>66</sub></b>	3200	1.1	0.1	-
<b>27 C<sub>33</sub> H<sub>68</sub></b>	3300	1.4	1.8	tr
<b>Identified (%)</b>	<b>-</b>	<b>84.5</b>	<b>85.9</b>	<b>90.9</b>

RI: Retention index; FRE-1: Farellones Road Extract-1; LPPE: Lo Prado Pass Extract; Farellones Road Extract-2: FRE-2; tr: trace amounts (< 0.03%).

In order to evaluate what happens when the epicuticular layer of the leaves area eliminated, the samples collected in Farellones underwent a second extraction (FRE-2) 5 minutes after the first extraction.

In this fraction, 90.9% of the compounds were identified. Surprisingly, the fraction showed 3-(Pentadec-10-enyl)-catechol (**1**) as the main component (67.5%), monoterpenes and sesquiterpenes not detected in the fraction obtained from the first extraction.

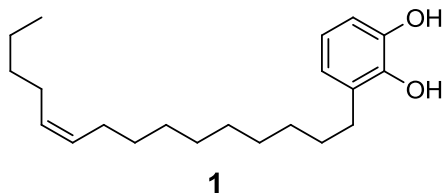
The existence of a lipid layer composed mainly of linear hydrocarbons agrees with the published data from electron microscopy (Montenegro

*et al.*, 1984), which show an epicuticular lipid layer model classified as smooth, within the 23 known examples (Barthlott *et al.*, 1998).

These results indicate that the smooth cuticle hydrocarbon layer of the leaves function as a lipophylic barrier that controls allergen release.

In addition, the two Litre leaf samples showing different amounts of the allergen supports the rural myths that certain types of Litre are more “aggressive” to sensitive people and cause more intense dermatitis due to the greater amounts of 3-(Pentadec-10-enyl)-catechol (**1**) on the surface of the leaves.

The lipophilicity of 3-(Pentadec-10-enyl)-catechol (**1**) was estimated theoretically at 8.12 (Cheng *et al.*, 2007). This lipophilicity ensures a great affinity of the hydrophobic region of the molecule to the epicuticular wax component, leaving the polar region corresponding to the catechol group exposed at the surface. In the same way, the aliphatic chain of (**1**) helps the hapten to bind with the cytoplasmatic membrane of epidermal cells, allowing the allergen to react with membrane proteins and/or to be internalized by skin cells for activation (Kalish, 1991).



## EXPERIMENTAL

### General

Limonene;  $\alpha$ -pinene;  $\beta$ -pinene; p-cymene and menthol were purchased from Sigma-Aldrich (St. Louis, MO, USA). The epicuticular component analysis was performed using gas chromatography (GC) and gas chromatography/mass spectroscopy (GC/MS). Qualitative analysis was performed using a Hewlett-Packard 5891 gas chromatograph linked to a Hewlett-Packard 5972 mass spectrometric detector with an integrated data system (Hewlett Packard, Palo Alto, CA, USA); quantitative analysis was carried out using a Shimadzu GC-9A gas chromatograph fitted with a FID-9 detector (Shimadzu Corporation, Kyoto, Japan). The same capillary column (SPB-5, film thickness 0.25  $\mu$ m, 30m x 0.25 mm, Supelco, Deerfield IL, USA) was used in both instruments.

### Plant material

Representative leaves samples of *Lithrea caustica* (Mol) Hooker & Arnott, (Anacardiaceae), were collected in November 2009 (spring) from two locations: 1) Región Metropolitana, Lo Prado Pass (LPP) (33° 10' S, 70° O); 2) Región Metropolitana, Farellones Road (FR) (31° 21,5' S, 70° 21' S). Voucher specimens were deposited in the Herbarium of the National Natural History Museum, Santiago, Chile.

### Epicuticular components extraction and analysis

Leaves of each sample of *L. caustica* (100 g) were extracted by dipping the fresh plan material in 0.6 L of

cold  $\text{CH}_2\text{Cl}_2$  for 30 s, to assure the total and selective extraction of the epicuticular components. In the case of the plan material collected in Farellones Road, the extraction was repeated twice. The epicuticular component analysis was performed by gas chromatography (GC) and gas chromatography/mass spectroscopy (GC/MS) using the instrumentation described above. The operating conditions were as follows: on-column injection; injector temperature, 250° C; detector temperature, 280° C; carrier gas, He at 1.25 ml/min; oven temperature program: 35° C for 5 min, increase to 260° C at 5° C/min, and then 260° C for 5 min. The mass detector ionization employed an electron impact of 70 eV. Recording conditions employed a scan time of 1.5 s and a mass range of 50 to 500 amu. Compounds in the chromatograms were identified by comparison of their mass spectra with those in the NIST08 library database, literature (Gambaro *et al.*, 1986) and by comparison of their retention index with those reported in the literature (Adams, 2007), for the same type of column or those of commercial standards, when available.

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