New 5-Alkenyl Resorcinols from *Lithraea molleoides* (Vell). Eng.

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**Abstract**

*Lithraea molleoides* (Anacardiaceae) is a tree that grows in South America including Southern Brazil, Southern, and Eastern Bolivia, Southern Paraguay, Northern, and Central Argentina. Infusions, decoctions, or tinctures from its aerial parts (leaves, buds, and young stems) are employed in ethnomedicine mainly against respiratory, and digestive inflammations and illnesses. Antibacterial, antiviral, antioxidant, anti-inflammatory, and antinociceptive activities, among others, have been reported for *L. molleoides*. Many of its biological activities have been associated with the reported presence of 5-alkenyl resorcinols. Alkyl/alkenyl catechols and alkyl/alkenyl resorcinols are very common in members of the Anacardiaceae family and several activities have been attributed to them. This work describes the isolation and the structural elucidation of three new 5-alkenyl resorcinols isolated from *Lithraea molleoides* reported in nature for the first time.

**Keywords**

*Lithraea molleoides*, 5-Alkenyl Resorcinol,
(Z,Z)-5-(Pentadeca-6,9-Dienyl)-Resorcinol,
(Z,Z)-5-(Trideca-5,8-Dienyl)-Resorcinol,
(Z)-5-(Heptadec-6-Enyl)-Resorcinol

1. Introduction

The Anacardiaceae family comprises a great variety of medicinal plants from
which many biologically active substances have been isolated. Alkyl/alkenyl catechols and alkyl/alkenyl resorcinols, also phenolic lipids, are very common in members of this family and several activities have been attributed to them. The antifungal activity of 5-(12-heptadecenyl)-resorcinol and the anti-inflammatory activity of 5-(11’Z-heptadecenyl)-resorcinol and 5-(8’Z,11’Z-heptadecadienyl)-resorcinol, all compounds isolated from the peel of Mangifera indica L. (Anacardiaceae), have been informed [1]. Also, the resorcinol 5-((8Z,11Z,14Z)-hexatriaconta-8,11,14-trienyl) benzene-1,3-diol has been reported for its cytotoxic and apoptotic effects [2]. Lithraea caustica (Anacardiaceae) extract, characterized by the presence of long-chain catechols, has been demonstrated to promote an antitumoral response against M16 melanoma [3].

Lithraea molleoides (Vell.) Engl. (Anacardiaceae) is a tree that grows in Southern Brazil, Southern, and Eastern Bolivia, Southern Paraguay, Northern, and Central Argentina. In Argentina, it is a dominant orophilic species in the biogeographical Semi-arid Chacoan region. In the Paranaense and Humid Chacoan rainforests, L. molleoides is a member of the secondary succession community [4] [5] where is commonly named “chichita”, “molle dulce” or “molle de Córdoba”. Infusions, decoctions or tinctures from the aerial parts (leaves, buds, and young stems) are used by rural people of these countries for its antiarthritic, hemostatic, diuretic, and tonic properties [6] as well as used for the treatment of respiratory diseases [7] and digestive inflammations illnesses [8]. Previous investigations on different extracts of L. molleoides have reported antiviral [9], antimicrobial [10], anti-inflammatory [11], anti-ulcerogenic [12], antinociceptive [13], and antibacterial [14] activities.

The resorcinol 1,3-dihydroxy-5-(tridec-4’,7’-dienyl)-benzene, is the most cited compound of L. molleoides as responsible for their biological activities. It has been isolated from this plant and showed cytotoxicity on human hepatocellular carcinoma cell line [15] as well as apoptosis induction [16]. The anti-inflammatory activity [8], the antioxidant and antiproliferative activity on tumor lymphocytes, and immunostimulant activity on normal lymphocytes [17] of the dichloromethane extract of L. molleoides were reported and identified this compound as the major component. The same compound has been identified in the ethanolic extract of L. molleoides and showed antibacterial activity against Proteus mirabilis [18]; it has been proposed as an effective tyrosinase inhibitor [19] to use in food preservation. Moreover, 13 carbon alkenyl resorcinols chains obtained from this species have shown nematocidal activity [20].

This work aims to isolate the minor resorcinols present in the L. molleoides dichloromethane extract and chemically identify them.

2. Materials and Methods

2.1. Plant Material

Leaves of Lithraea molleoides were collected in the district of Burruyacú, Sierra de Medina, province of Tucumán, Argentina, and botanically identified by Ph.D. Graciela Ponessa. Vouchers specimens are deposited in the Herbarium
2.2. Plant Extraction and Compound Isolation

Leaves of *L. molleoides* (70 g) were overnight macerated (6 × 150 ml) with dichloromethane (Cl₂CH₂) at room temperature. After vacuum filtration, the Cl₂CH₂ extracts were joined and taken to dryness under reduced pressure yielding 3.95 g of dried extract. The dry extract was separated on a Sephadex LH-20 column (50 cm × 5 cm) using a gradient of Cl₂CH₂ and methanol (MeOH) as solvents according to López et al. [16] obtaining 24 fractions. The fractions were monitored by TLC on Silicagel F₂₅₄ plates using Cl₂CH₂-MeOH (95:5) as the mobile phase. Five fractions containing resorcinol derivatives were obtained.

The compounds were isolated from the resorcinol fractions by HPLC-UV DAD performed with a Varian® 9000 instrument using a diode array detector. An RP18 column (Gemini® 5 μm, 150 mm × 4.6 mm) and a mobile phase constituted by solvent A: H₂O/AcOH (98:2) and solvent B: MeOH/AcOH (98:2) was used. A gradient consisting in 70% B to 100% B in 30 minutes was applied. The flow rate was 1.2 ml/min. Eleven eluates were collected and taken to dryness in a Savant® concentrator. Each compound was numbered in order of increasing elution.

2.3. Compound Identification

Mass spectra were determined in an ESI Bruker microTOF-Q II™spectrometer. 1H and 13C-NMR spectra, homo- and heteronuclear correlation spectroscopy and NOESY experiments were recorded at ambient temperature in chloroform-d₆ (CDCl₃) with a Magneto Bruker Ultra Shield spectrometer operated at 600.13 MHz and 150.91 MHz for 1H and 13C nucleus respectively. Chemical shifts are expressed in ppm (relative to the solvent). Coupling constants (J) are in Hz.

3. Results and Discussion

Five 5-alkenyl resorcinols were obtained from the dichloromethane extract of *L. molleoides* (supplementary data) and after structural elucidation, they were identified as:

(Z,Z)-5-(trideca-4,7-dienyl)-resorcinol 3 [20] 1H NMR (600 MHz): δ (ppm): 6.27 (2H, d, J = 2.3 Hz), 6.20 (1H, t, J = 2.3 Hz), 5.44 - 5.34 (4H, m), 2.79 (2H, t, J = 5.9 Hz), 2.51 (2H, t, J = 7.7 Hz), 2.10 (2H, q, J = 7.2 Hz), 2.06 (2H, q, J = 7.1 Hz), 1.66 (2H, quint., J = 7.6 Hz), 1.40 - 1.27 (6H, m), 0.90 (3H, t, J = 6.8 Hz). 13C NMR (150.903 MHz): δ (ppm): 156.63, 145.73, 130.40, 129.46, 128.66, 108.08, 100.27, 35.29, 31.51, 30.91, 29.32, 27.20, 26.76, 25.67, 22.57, 14.07.

(Z)-5-(trideca-4-enyl)-resorcinol 5 [20] 1H NMR (600 MHz): δ (ppm): 6.27 (2H, d, J = 2.3 Hz), 6.20 (1H, t, J = 2.3 Hz), 5.44 - 5.36 (2H, m), 2.52 (2H, t, J = 7.7 Hz), 2.08 (2H, q, J = 7.0 Hz), 2.02 (2H, q, J = 6.9 Hz), 1.66 (2H, quint., J = 7.6 Hz), 1.31 - 1.26 (14H, m), 0.90 (3H, t, J = 6.7 Hz). 13C NMR (150.903 MHz): δ
(ppm): 156.63, 145.78, 130.60, 129.08, 108.07, 100.25, 35.32, 31.89, 31.01, 29.73, 29.50, 29.32, 29.29, 27.28, 26.78, 22.65, 14.06.

(Z,Z)-5-(pentadeca-6,9-dienyl)-resorcinol 6 

\[ { }^1 \text{H NMR (600 MHz): } \delta (\text{ppm}): 6.26 (2H, d, J = 2.3 \text{ Hz}), 6.20 (1H, t, J = 2.3 \text{ Hz}), 5.40 - 5.33 (4H, m), 2.79 (2H, t, J = 6.8 \text{ Hz}), 2.51 (2H, t, J = 7.8 \text{ Hz}), 2.08 (4H, m), 1.61 (2H, m), 1.40 - 1.28 (10H, m), 0.90 (3H, t, J = 6.8 \text{ Hz}). \]

\[ { }^{13} \text{C NMR (150.903 MHz): } \delta (\text{ppm}): 156.61, 145.98, 130.26, 129.14, 127.90, 108.00, 100.18, 35.77, 31.55, 30.91, 29.50, 29.33, 28.92, 27.21, 27.14, 25.64, 22.55, 14.03. \]

Table 1 shows the spectroscopic data of compounds 6, 9 and 11 (Figure 1).

Analysis of \(^1\text{H}\) and \(^{13}\text{C}\) NMR spectra of the isolated compounds indicates the presence of a resorcinol subunit in all of them with different side chains with various insaturation degrees (Table 1). Negative HRMS (ESI) showed the unprotonated molecular ion at \(m/z\) 315 for compound 6. The corresponding molecular formula \(\text{C}_{21}\text{H}_{32}\text{O}_2\) and the overlapping signals of four olefinic protons \(\delta_H 5.37 - 5.40\) suggested a 15-carbon side chain with two double bonds. HSQC spectra allowed the assignment of the multiplicity of all protonated carbons, which confirmed that \(\text{C}_{13}\) side chain was linear. HMBC data established the position of the double bonds at \(\Delta^6\) and \(\Delta^9\) with a bisallylic methylene group at \(\delta_H 2.79\) and \(\delta_C 25.64\) ppm. The proton signals of the double bond are complex and appear overlapped, unable to determine the coupling constants that would allow the assignment of the double bond configuration. However, the \(^{13}\text{C}\) NMR resonances \(\delta_C 27.14\) and \(27.21\) ppm for the two allylic methylenes at position 5' and 11', and \(\delta_C 25.64\) ppm for the bisallylic methylene C-8' are also in agreement with literature data reported for other resorcinols with Z geometry [21]. Taking into account the spectroscopic data, compound 6 was identified as (Z,Z)-5-(pentadeca-6,9-dienyl)-resorcinol.

Positive HRMS (ESI) of compound 9 showed molecular ion+Na at \(m/z\) 311 that correspond to a molecular formula \(\text{C}_{19}\text{H}_{28}\text{O}_2\). Their \(^1\text{H}\) and \(^{13}\text{C}\) NMR spectra were similar to those of 6 and indicated \(\text{C}_{13}\) linear alkenyl resorcinol. \(^1\text{H}\) NMR spectra revealed resonance of one bisallylic methylene group, suggesting that the double bonds were separated by methylene group. HSQC and HMBC experiments allowed the assignment of all protonated carbons and showed that the double bonds are placed at \(\Delta^6\) and \(\Delta^8\). The \(^{13}\text{C}\) NMR resonances for the two allylic methylenes at position 4' and 10' have the same value \(\delta_C 27.21\) ppm and
Figure 1. Structural formulas of the 5-alkenyl resorcinols isolated from *L. molleoides*.

Table 1. $^{13}$C, $^1$H NMR, HSQC, and HMBC Data of Compounds 6, 9 and 11 in CD$_3$OD$^a$.

| position | $\delta$C | $\delta$H | HMBC | $\delta$C | $\delta$H | HMBC | $\delta$C | $\delta$H | HMBC |
|----------|-----------|-----------|------|-----------|-----------|------|-----------|-----------|------|
| 1        | 156.61    | 156.64    |      | 156.61    | 156.64    |      | 156.61    | 156.64    |      |
| 2        | 100.18    | 6.20      | 1, 3, 4, 6 | 100.13 | 6.19      | 1, 3, 4, 6 | 100.17 | 6.20 | 1, 3, 4, 6 |
| 3        | 156.61    | 156.64    |      | 156.61    | 156.64    |      | 156.61    | 156.64    |      |
| 4        | 108.03    | 6.26      | 2, 3, 6, 1' | 107.99 | 6.26      | 2, 3, 6, 1' | 108.60 | 6.26 | 2, 3, 6, 1' |
| 5        | 145.98    | 146.08    |      | 146.08    | 146.11    |      | 146.08    | 146.11    |      |
| 6        | 100.18    | 6.26      | 1, 2, 4, 1' | 107.99 | 6.26      | 1, 2, 4, 1' | 108.60 | 6.26 | 1, 2, 4, 1' |
| 1'       | 35.77     | 2.51      | 4, 5, 6, 2', 3' | 35.81 | 2.50      | 4, 5, 6, 2', 3' | 35.79 | 2.54 | 2' |
| 2'       | 30.91     | 1.61      | 5, 1', 3', 4' | 31.03 | 1.60      | 1', 3' | 30.98 | 1.61 | 1', 3' |
| 3'       | 28.92     | 1.37      | 4', 2' | 29.23 | 1.33      | 2', 4' | 29.20 | 1.25 | 1.38 | 2', 3' |
| 4'       | 29.33     | 1.37      | 5'    | 27.21 | 2.06      | 3', 5', 6', 29.74 | 1.25 | 1.38 | 5' |
| 5'       | 27.14$^a$ | 2.08      | 6', 7', 9', 10', 4' | 130.05$^b$ | 5.33      | 4', 7', 27.19$^a$ | 2.04 | 4', 6' |
| 6'       | 130.26$^b$ | 5.40     | 8'    | 128.02$^c$ | 5.34      | 4', 7' | 129.81$^b$ | 5.40 | 5 |
| 7'       | 127.90$^c$ | 5.37     | 8'    | 25.64 | 2.79      | 5', 6', 8', 9' | 129.97$^b$ | 5.37 | 8' |
| 8'       | 25.64     | 2.79      | 6', 7', 9', 10' | 127.94$^c$ | 5.37 | 7', 10' | 27.22$^d$ | 2.04 | 7', 9' |
| 9'       | 128.14$^b$ | 5.37     | 8'    | 130.13$^b$ | 5.38      | 7', 10' | 29.76 | 1.25 | 1.38 |
| 10'      | 129.95$^c$ | 5.40     | 8'    | 27.21 | 2.06      | 8', 9', 11', 29.22 | 1.25 | 1.38 |
| 11'      | 27.21$^d$ | 2.08      | 6', 7', 9', 10', 12', 13' | 31.53 | 1.31      | 10', 11' | 29.31 | 1.25 | 1.38 |
| 12'      | 29.50     | 1.33      |      | 22.58 | 1.32      | 13'    | 29.29 | 1.25 | 1.38 |
| 13'      | 31.55     | 1.34      |      | 14.08 | 0.90      | 11', 12' | 29.37 | 1.25 | 1.38 |
| 14'      | 22.55     | 1.33      | 15', 13' |      | 29.50 | 1.25 | 1.38 |
| 15'      | 14.03     | 0.90      | 14', 13' |      | 31.89 | 1.31 |      |
| 16'      | {}        | {}        | {}   | 22.66 | 1.31      | 15', 17' |      |
| 17'      | 14.05     | 0.90      | 15', 16' |      |       |

$^a$Correlations between $^1$H and $^{13}$C based on HSQC spectra. $^{b,c,d}$Assignments interchangeable.
are in agreement with Z configuration. Based on the spectroscopic data compound 9 was identified as (Z,Z)-5-(trideca-5,8-dienyl)-resorcinol.

Their 1H and 13C NMR spectra of 11 were similar to those of 6 and indicated C17 linear alkenyl resorcinol. Negative HRMS (ESI) showed the unprotonated molecular ion at m/z 345 that corresponds to a molecular formula C23H38O2. A multiplet integrating for two protons (δH 5.40 - 5.37 ppm) is observed in 1H NMR spectrum. HSQC and HMBC experiments unambiguously allowed the assignment of a Δ6' double bond. 13C NMR chemical shifts of 27.19 and 27.22 ppm for allylic C-5’ and C-8’ confirmed the Z geometry in compound 11. Based on the spectroscopic data compound 11 was identified as (Z)-5-(heptadec-6-enyl)-resorcinol.

The major alkenyl resorcinols 3 and 5 were previously isolated from L. molleoides and associated with nematicidal [20] and cytotoxic activities [15] [16]. Compound 2 was a mixture of isomers (Z,Z,Z)-5-(trideca-4,7,10-trienyl)-resorcinol and (Z,Z,E)-5-(trideca-4,7,10-trienyl)-resorcinol that could not be separated by the HPLC system used and these had already been reported by Valcic et al. [20].

This is the first report in the nature of the 5-alkenyl resorcinols 6, 9 and 11.

The knowledge of the bioactive compounds present in extracts obtained from medicinal plants is of great importance and contributes to the characterization of species with medicinal potential. Over the last decades, natural products have been shown to be very varied sources of compounds that served to search for new molecules with biological activities of importance in human medicine [22]. Given previous reports and the promissory activity of 5-alkyl/alkenyl resorcinols further studies must be conducted to test the biological activity of the new alkenyl resorcinols isolated from L. molleoides.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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