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1,3-dihydroxy-5-(tridec-4′,7′-dienyl)benzene: a new cytotoxic compound from *Lithraea molleoides*

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Abstract

A dichloromethane extract from the leaves of *Lithraea molleoides* (Anacardiaceae), an argentine medicinal plant, showed cytotoxicity on human hepatocellular carcinoma cell line. Bioassay guided fractionation of this extract led to the isolation of a new active 5-alkyl resorcinol: 1,3-dihydroxy-5-(tridec-4′,7′-dienyl)benzene. Chemical structure was established based on spectroscopic data (UV, IR, MS, ^{1}H-NMR, ^{13}C-NMR, COSY). This compound presented cytotoxic activity on 3 human tumoral cell lines: hepatocellular carcinoma cell line-Hep G2 ($IC_{50} \pm SD$ of $68 \pm 2\,\mu M$), mucoepidermoid pulmonary carcinoma cell line-H292 ($IC_{50} \pm SD$ of $63 \pm 5\,\mu M$) and mammary gland adenocarcinoma cell line -MCF7 ($IC_{50} \pm SD$ of 147 ± 5).

Keywords: *Lithraea molleoides*; Cytotoxic activity; 5-alkylresorcinol; 1,3-dihydroxy-5-(tridec-4′,7′-dienyl)benzene

Introduction

The search for new cytotoxic agents, from natural sources continues promoting the collaboration among scientists worldwide. The ethnopharmacological knowledge is helpful to explore plants with potential cytotoxic activity.

Anacardiaceae family comprises many medicinal species from which a number of biologically active substances such as phenol derivatives have been isolated. Among them, 5-alkyl resorcinols present antibacterial, fungicidal and cytotoxic activities, which are related to their strong interaction with biological membranes (Kozubek et al., 2001; Chaturvedula et al., 2002).

Lithraea molleoides (Vell.) Engl. (Anacardiaceae), commonly known as ''chichita'' or ''molle de Córdoba'', is a tree that grows in South America, specially in Argentina, Brasil and Uruguay and is well known by rural people of these countries as: antiarthritic, hemostatic, diuretic, tonic and useful for the treatment of respiratory diseases (Toursarkissian, 1980; Muñoz, 1990).

Previous investigations on different extracts of this plant have reported antiviral (Kott et al., 1999) and antimicrobial (Penna et al., 2001) activities. Allergic contact dermatitis has been reported and four allergenic compounds have been characterized as pyrocatechol

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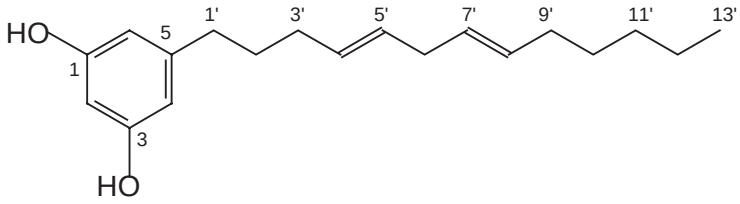

Fig. 1. Formula of 1,3-dihydroxy-5-(tridec-4′,7′-dienyl)benzene (**1**).

derivatives, but not fully identified, from different extracts of this plant and from *L. brasiliensis* (Alé et al., 1997). Cytotoxic activity has been reported for the methanol extract of *L. molleoides* on Hep G2 cells ($IC_{50} \pm SD$ of $244 \pm 25\,\mu g/ml$) (Ruffa et al., 2002).

Further investigation of the dichloromethane extract of *L. molleoides* on Hep G2 cells showed that this extract is more cytotoxic ($IC_{50} \pm SD$ of $117 \pm 6\,\mu g/ml$) and activity-guided fractionation of this extract has led to the isolation of a pure bioactive compound, a new cytotoxic 5-alkyl resorcinol derivative: 1,3-dihydroxy-5-(tridec-4′,7′-dienyl)benzene (**1**) (Fig. 1).

Materials and methods

Plant material

L. molleoides was collected in Entre Ríos Province, Argentina and was identified by Ing. Juan de D. Muñoz. A voucher specimen (Herbarium Muñoz No. 1714) is deposited at the Facultad de Ciencias Agropecuarias de Entre Ríos, Argentina.

Isolation and identification

The dried leaves of *L. molleoides* (800 g) were extracted overnight with CH_2Cl_2 (3×3500 l) at room temperature. The concentration of the extract under reduced pressure yielded a dark syrupous extract (27.3 g). A portion (16.7 g) of this extract was chromatographed on a Sephadex LH-20 column (45 cm × 30 cm) using CH_2Cl_2 and CH_2Cl_2:MeOH (1:1) as solvents yielding 7 fraction (Fr.).

The eluates were monitored by TLC on silicagel (Merck) plates using $CHCl_3$:MeOH (95:5) as mobile phase. Fr 6 and 7 were pooled and purified passing it through another Sephadex LH-20 column (45 cm × 30 cm) using toluene:CH_2Cl_2 (7:3 and 1:1) and MeOH as solvent. The MeOH fraction was purified by HPLC using a ODS column with 70–100% MeOH:HOAc (98:2) in 30 min. Compound **1** (6 mg) was identified as

$$-\text{dihydroxy} - 5 - (\text{tridec} - 4', 7' - \text{dienyl})\text{benzene} : \quad (1)$$

Table 1. ^{1}HNMR (500 MHz), ^{13}CNMR (125 MHz) data of compound (**1**) 1,3-dihydroxy-5-(tridec-4′,7′-dienyl)benzene (**1**)[a]

H	δ_H (ppm)	C	δ_C (ppm)
1′	2.50 t (7.5)	1′	35.3
2′	1.63 m	2′	29.3
3′	2.09 m	3′	31.5
4′	5.38 m	4′	129.5
5′	5.35 m	5′	128.7
6′	2.76 t (5.9)	6′	27.2
7′	5.35 m	7′	127.8
8′	5.38 m	8′	130.4
9′	2.04 m	9′	26.8
10′	1.35 m	10′	25.7
11′	1.30 m	11′	30.9
12′	1.30 m	12′	22.6
13′	0.89 t (7.0)	13′	14.1
1		1	156.7
2	6.17 s	2	103.0
3		3	156.7
4	6.24 s	4	108.0
5		5	145.7
6	6.24 s	6	108.7

[a]Chemical shifts (δ) in ppm relative to TMS, J values (in Hz) in parentheses.

($C_{19}O_2H_{28}$), colorless oil, UV λ MeOH max nm ($\log \varepsilon$): 274 (0.55). IR (film) max cm^{-1}: 3460 (OH), 3020 (C=C), 2960, 2880, 1620 and 1600 (Ar), 1480, 1360, 1290 (C–O), 835, 785, 740 (Ar). EIMS: m/z (rel. int): 289 [M + 1] (1.5), 288 [M +] (6.7), 271[M + 1-H_2O], 217 (5.8), 203 [M-85] (4.2), 189 (5), 176 (8.8), 163 [M-125] (23.4), 149 (8), 136 (9), 124 (100), 81 (12.9), 77 (14.1). ^{1}H-NMR (500 MHz), ^{13}C-NMR (125 MHz) and ^{1}H–^{1}H COSY data are shown in Table 1.

Human tumoral cell lines

Hep G2 cells derived from a hepatocellular carcinoma (ATCC-HB 8065); MCF7 cells obtained from a mammary gland adenocarcinoma (ATCC-HTB-22) and H292 cells derived from a mucoepidermoid pulmonary carcinoma (ATCC CRL-1848) were cultured with MEM (for Hep G2 and MCF7) or RPMI (for H292) medium supplemented with 10% foetal bovine

serum (FBS), glutamine 2 mM, sodium bicarbonate 2.25 g/l (for H292) or 1.5 g/l (for Hep G2 and MCF7), non-essential aminoacids 1 mM and 100 units/ml of penicillin G sodium–100 μg/ml of streptomycin sulfate, sodium pyruvate 1.0 mM (except for H292), bovine insulin 0.01 mg/ml (only for MCF7); and incubated at 37 °C in a humidified 5% CO_2 atmosphere.

In vitro cytotoxicity assay

Extract, Fr., and the compound were suspended at 10 mg/ml in DMSO:H_2O (1:5). For the microtitration assay, cells in a log-growth phase were incubated with different dilutions of the sample for 48 h; at that time the samples were replaced by fresh growth medium for further 24 h (Ruffa et al., 2002). Then the cell viability was evaluated by MTT assay (Freshney, 2000). The IC_{50} (inhibitory concentration 50%) and SD (standard deviation for 95% confidence), determined as the extract/Fr/compound concentration required to reduce the cell viability to half the control value, was analyzed by linear regression from the curve of the cell proliferation vs. the sample concentrations. In each assay doxorubicin hydrocloride (Gador) was included as a positive control.

Results and discussion

Looking for new cytotoxic compounds from Argentine medicinal plants, dichloromethane extract of *L. molleoides* was screened on Hep G2 cell line showing an $IC_{50} \pm$ SD of 117 ± 6 μg/ml. Thus, the bioassay-guided fractionation of this extract was performed. The IC_{50} were determined for all the fractions that showed cytotoxic activity. Fr 6 and 7 showed the lowest IC_{50} (32 and 34 μg/ml, respectively) against this cells; a major compound was found in these fractions.

The isolated compound showed the characteristic UV profile of an *m*-diphenol. IR spectra showed the presence of an aryl hydroxy group, and aliphatic bands for C–C, C=C and C–O were also present.

The molecular formula was established as ($C_{19}O_2H_{28}$), with a [M+] of 288.40 (calculated 288.43). It showed a base peak at 124, corresponding to the molecular formula $C_7H_8O_2$ (calculated 124.13) characteristic of diphenol derivatives. The presence of an ion at 203.10 (4.3%, M-85) corresponding to the loss of C_6H_{13} and that at 163 (23.40%, M-125) corresponding to the loss of C_9H_{17}, indicated the presence of a 13 carbon side chain with two double bonds. The presence in the ^{1}HNMR spectrum (Table 1) of one proton singlet at 6.17 and 2 proton singlet at 6.24 indicated a 1,3-dihydroxy substituted aromatic ring. This was confirmed by the presence of four signals in the ^{13}CNMR spectrum (Table 1) at 156.7 for C1 and C3, at 108.7 for C4 and C6, at 103.0 for C2 and at 108.0 for C4. This fact and the analysis of ^{1}H–^{1}H COSY led us to identify the new compound as 1,3-dihydroxy-5-(tridec-4′,7′-dienyl)-benzene.

This compound was assayed for cytotoxic activity on three human tumoral cell lines and it was active in all of them in a dose dependant manner. Hep G2 ($IC_{50} \pm$ SD of 68 ± 2 μM) and H292 cells ($IC_{50} \pm$ SD of 63 ± 5 μM) were more susceptible to this compound than the MCF7 cells ($IC_{50} \pm$ SD of 147 ± 5 μM).

The isolated compound presented cytotoxic activity in the *in vitro* assay; from these results and the activities of other 5-alkyl resorcinol reported previously (Kozubek, 1999) we could infer that it may have some kind of antitumoral activity. Nevertheless, further studies should be conducted to confirm this hypothesis. Taking into account the allergic potential of compounds with the structure described, side effects can be expected.

Catechol derivatives have been reported previously from the genus *Lithraea* (Gambaro et al., 1986; Alé et al., 1997), but this is the first time that a 5-alkyl resorcinol compound is reported.

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References

Alé, S., Ferreira, F., González, G., Epstein, W., 1997. Allergic contact dermatitis caused by *Lithraea molleoides* and *Lithraea brasiliensis*: identification and characterization of the responsible allergens. Am. J. Contact Derm. 8 (3), 144–147.

Chaturvedula, V.S.P., Schilling, J.K., Miller, J.S., Andriantsiferana, M.R., Rasamison, V.E., Kingston, D.G.I., 2002. New cytotoxic bis 5-alkylresorcinol derivatives from the leaves of *Oncostemon bojeraianum* from the Madagascar rainforest. J. Nat. Prod. 65 (11), 1627–1632.

Freshney, R.I., 2000. Cytotoxicity. In: Culture on animal cells. A manual of basic technique, 4th Ed.. Wiley-Liss, USA, pp. 329–345.

Gambaro, V., Chamy, M., von Brand, E., Garbarino, J., 1986. 3-(pentadec-10-enyl) catechol. A new allergenic compound from *Lithraea caustica* (Anacardiaceae). Planta Med. 20 (1), 2.

Kott, V., Barbini, L., Cruañes, M.C., Muñoz J de, D., Cruañes, J., Vivot, et al., 1999. Antiviral activity in Argentine medicinal plants. J. Ethnopharmacol. 64, 79–84.

Kozubek, A., 1999. Resorcinolic lipids, the natural non-isoprenoid phenolic amphiphiles and their biological activity. Chem. Rev. 99, 1–25.

Kozubek, A., Zarnowski, R., Stasiuk, M., Gubernator, J., 2001. Natural amphiphilic phenols as bioactive compounds. Cell. Biol. Mol. Lett. 6, 351–355.

Muñoz, J., 1990. Usos principales de las especies de Anacardiaceae, particularmente del Paraguay. Candollea 45, 671–680.

Penna, C., Marino, S., Vivot, E., Cruañes, M.C., Muñoz J de, D., Cruañes, J., et al., 2001. Antimicrobial activity of Argentine plants used in treatment of infectious diseases. Isolation of active compounds from *Sebastiania brasiliensis*. J. Ethnopharmacol. 77, 37–40.

Ruffa, M.J., Ferraro, G., Wagner, M.L., Calcagno, M.L., Campos, R.H., Cavallaro, L., 2002. Cytotoxic effect of Argentine medicinal plant extracts on human hepatocellular carcinoma cell line. J. Ethnopharmacol. 79, 335–339.

Toursarkissian, M., 1980. In: Plantas Medicinales de la Argentina Ed Hemisferio Sur, Buenos Aires. P 4.