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Original Article

Pentacyclic triterpenes from *Cecropia telenitida* with immunomodulatory activity on dendritic cells

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ABSTRACT

Pentacyclic triterpenes are a large family of plant metabolites that exhibit a wide array of biological activities. The genus *Cecropia*, which encompasses many plant species, has been used as traditional medicine for the treatment of inflammatory diseases and is known to produce many active pentacyclic triterpenes. In this study we investigated the chemical composition of a pentacyclic triterpene fraction from the roots of *Cecropia telenitida* Cuatrec., Urticaceae. A novel compound, which we termed yarumic acid, and four known molecules (serjanic acid, spergulagenic acid A, 20-hydroxy-ursolic acid and goreishic acid I) were isolated and characterised. In a dendritic cell (DC)-based assay, we demonstrated that non-toxic doses of these pentacyclic triterpenes inhibited the secretion of at least one of the proinflammatory cytokines tested (IL-1β, IL-12p40, IL-12p70, TNF-α). Spergulagenic acid A also inhibited nitric oxide production in lipopolysaccharide-stimulated dendritic cell. Serjanic acid and spergulagenic acid A, which were the most potent abundant compounds in the pentacyclic triterpene fraction, showed the most activity in the dendritic cell-based assay. These results show that all pentacyclic triterpenes might contribute to the anti-inflammatory activities of *C. telenitida*. Moreover, yarumic acid as well as the four known pentacyclic triterpenes, can be exploited as potential immunomodulatory/anti-inflammatory agents.

Introduction

Pentacyclic triterpenes (PT) are secondary plant metabolites that are widely distributed in many species within the plant kingdom and therefore throughout the world. These metabolites are found in varying amounts in edible vegetables and fruits, making them important constituents of the human diet (Jäger et al., 2009; Laszczyk, 2009; Saleem, 2009; Siddique and Saleem, 2011). Consumption of this class of naturally occurring substances appears to be associated with the prevention of a variety of human diseases (Laszczyk, 2009; Siddique and Saleem, 2011). Phytochemical investigations of medicinal plants used in folk medicine have also consistently implicated PT as one of the most prominent chemical groups responsible for the pharmacological activity of the derived natural products (Poeckel and Werz, 2006; Ríos, 2010; Saleem,

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2009). *In vitro* and *in vivo* animal studies have demonstrated the potential of prototypical PT, such as lupeol and betulinic acid, as well as ursolic, boswellic and oleanolic acid derivatives, to prevent and/or treat a spectrum of diseases that range from cancer and infection to diabetes and rheumatic and cardiovascular diseases (Laszczyk, 2009; Sheng and Sun, 2011; Siddique and Saleem, 2011). Interestingly, most of PT studied thus far exhibit immunomodulatory activity, as they inhibit one or several pathways involved in the inflammatory/immune response (Ríos, 2010; Saleem, 2009). Therefore, modulation of the immune system is emerging as a central mechanism that explains, at least in part, the multi-targeting properties of PT.

Dendritic cells (DC) are a class of leucocytes that play an essential role in the initiation and regulation of the immune response. As the strongest antigen-presenting cell (APC), DC have a unique ability to activate naïve T cells and coordinate the subsequent adaptive response towards different pathways, including inflammatory responses (such as Th1, Th2, Th17 etc.) and regulatory responses (such as Tr1, Th3 and other regulatory T cells). Typically, tissue-resident DC are activated by mediators either produced by microbial pathogens (also known as pathogen-associated molecular patterns, PAMP) or delivered by dying/stressed cells (also known as danger-associated molecular patterns, DAMP) and respond by undergoing a differentiation process called maturation. Maturation allows DC to coordinate the upregulation of surface molecules associated with antigen presentation (such as MHC class II and costimulatory molecules) and cytokine/chemokine secretion with migration to lymphoid organs. In the lymphoid organs, maturing DC stimulate lymphocyte proliferation and differentiation (Steinman and Hemmi, 2006). Secretion of pro-inflammatory cytokines by DC appears to be a prominent event that initiates and sustains chronic inflammatory responses, making this cell type a potential target to develop strategies against chronic inflammatory diseases (Chan et al., 2012; Farkas and Kemény, 2012). Because the majority of clinically available anti-inflammatory/immunosuppressive drugs are potent inhibitors of cytokine production by DC (Hackstein and Thomson, 2004), natural and synthetic compounds that exhibit this inhibitory effect on DC are considered to be promising anti-inflammatory/immunosuppressive leads.

Cecropia, Urticaceae, is a neotropical genus frequently found in humid areas up to 2600 m above sea level. Species of *Cecropia* occur from Mexico to northern Argentina. In contrast, the distribution of *C. telenitida* (Franco-Roselli and Berg, 1997), retrieved from the Tropicos database (Missouri Botanical Garden), shows that this species is restricted to the central and northern sections of the Andes Mountains (Venezuela, Colombia, Ecuador and Peru). Despite the fact that *C. telenitida* has not been well studied with regards to its chemistry and bioactivity, the genus has a large record of phytochemical descriptions (Costa et al., 2011; Rocha et al., 2007; Schinella et al., 2008). Chlorogenic acid and isoorientin are two recognised secondary metabolites identified in the leaves of *C. obstusifolia* and *C. pachystachya*, whereas PT are broader chemotaxonomic markers in roots of the plants belonging to this genus (Andrade-Cetto and Vázquez, 2010). The principal ethnopharmacological applications of *Cecropia* are for inflammation and the treatment of type 2 diabetes (Alonso-Castro et al., 2008; Andrade-Cetto and Vázquez, 2010; Aragão et al., 2010; Revilla-Monsalve et al., 2007; Schinella et al., 2008). In our search for naturally occurring bioactive compounds from the Colombian flora, we initiated a phytochemical investigation of *Cecropia telenitida* (vernacular names Guarumo, Guarumbo or Yarumo Plateado). Our preliminary studies indicated the presence of abundant PT in the roots. We also examined the composition of a PT fraction from the roots of *Cecropia telenitida* and evaluated its immunomodulatory potential *in vitro* by using DC as a read out system. Our results showed the presence of a novel compound that we have termed yarumic acid **(1)**, along with other four known PT. The immunomodulatory activity of all of the isolated PT as well as the total PT fraction is also reported.

Materials and methods

General experimental procedures

Methanol, ethyl acetate and *n*-hexane were purchased from Merck (Darmstadt, Germany). Deionised water was obtained from a Milli-Q water purification system (Millipore, Bedford, MA, USA). Ammonium acetate and formic acid were purchased from Sigma-Aldrich Chemie (Deisenhofen, Germany). NMR experiments were performed on Bruker DRX-400 and 600 spectrometers (Bruker, Rheinstetten, Germany) equipped with an HX inverse probe (^{1}H NMR: 400 MHz; ^{13}C: 100 MHz; ^{1}H: 600 MHz; ^{13}C: 150 MHz). ^{1}H NMR, ^{13}C NMR, H-H correlation (COSY), 1D and 2D distortionless enhancement by polarisation transfer (DEPT), and heteronuclear multiple quantum/bond coherence/correlation (HMQC/HMBC) were recorded using standard Bruker pulse sequences. System control and data evaluation was performed with the XWINNMR software package, version 2.6 (Bruker, Rheinstetten, Germany). Chemical shifts (δ) are expressed in ppm. The centre of the peak for each deuterated solvent was used as an internal reference (CD_3OD δ 4.78 and 3.31, $CDCl_3$ δ 7.24, and DMSO-d_6 δ 2.5 ppm).

HPLC separations were performed on an Agilent 1200 Series HPLC system (Agilent, Waldbronn, Germany). The separations were carried out on a C18 analytical column (250 mm × 4.6 mm, 5 µm; Agilent Zorbax Bonus RP) at 35°C and a C18 preparative Supelcocolumn (250 mm × 21.2 mm, 5 µm). The mobile phase (water: methanol) was comprised of 70 to 100% of methanol from 0 to 10 min and isocratic 100% methanol from 10 to 15 min. Ammonium acetate (5 mM) was added to the mobile phase to promote the negative ionisation mode. The flow rate was set at 0.8 ml/min. System control and data evaluation was performed using the Agilent LC/MSD Chemstation software package. APCI/MS was performed by means of the LC/MSD ion trap (Agilent, Waldbronn, Germany). The instrumental parameters of APCI were as follows: capillary voltage +2000; corona, 10000 nA; nebuliser pressure, 60 psi; dry gas flow, 5 l/min; dry gas temperature, 350°C; vaporiser temp, 400°C and skimmer voltage, -40V. The instrument was operated in negative mode. The full mass scanning range was *m/z*

400 to 600. To avoid the interference from background, the selected ion monitoring (SIM) mode was also used for some of the samples.

Plant material, extraction of triterpene fractions and isolation of compounds

The roots of *Cecropia telenitida* Cuatrec., Urticaceae, were obtained in La Ceja, Antioquia (parcel "el Yarumo"); the specimens were deposited in the Universidad de Antioquia herbarium (voucher: Alzate 2870, HUA). Air-dried roots of *C. telenitida* (2 kg) were cleaned with *n*-hexane and ethanol and then extracted with ethyl acetate. The ethyl acetate extract was concentrated (112 g), diluted in methanol and purified by Sephadex LH-20 using medium pressure liquid chromatography to eliminate molecules with a high molecular weight, such as polymers. The fractions containing triterpenes (1% yield; detection sulphuric acid/anisaldehyde reagent) were pooled and used for analytical and biological assays (20 g). Conventional isolations from preparative HPLC were used to purify the molecules. Serjanic (**2**) (1,450 g, 0.07% yield) and spergulagenic A (**3**) acids (0,700 g, 0.03% yield) were the most abundant triterpenes from *C. telenitida* roots, followed by yarumic acid (**1**) (200 mg, 0.01% yield). Other triterpenes were present at less than 5 mg (20-hydroxy-ursolic acid (4) and goreishic acid I (5)). Stock solutions of purified triterpenes and total triterpenefraction were prepared in DMSO (2 mg/ml).

Mice and bone marrow cell preparation

C57BL/6 mice obtained from Charles River Laboratories (Wilmington, MA) and were bred and housed under barrier conditions at the SPF animal facility of the Sede de Investigacion Universitaria (SIU) at Universidad de Antioquia. All procedures involving animals were approved by the institutional ethical committee. Serological and microbiological tests were periodically performed on the mice colony, and results showed that the mice were pathogen-free. To obtain total bone marrow cells, female C57BL/6 mice (8-12 weeks old) were sacrificed, and the femurs and tibiae were aseptically removed. A tuberculin syringe was then used to flush the bone marrow cell content and to obtain a homogeneous cell suspension.

Dendritic cells (DC) generation and treatments

DC were generated from bone marrow precursors according to the method reported by Lutz et al. (Lutz et al., 1999), with minor modifications. Briefly, 2×10^5 cells/ml bone marrow cells were washed and cultured in RPMI 1640 (Glutamax, GIBCO, USA) supplemented with 10 mM HEPES, 2 mM L-glutamine, 10% FBS, 50 µM β-mercaptoethanol and recombinant murine granulocyte/macrophage colony-stimulating factor (mGM-CSF, 200 U/ml). Cultures were incubated in 5% CO_2 atmosphere at 37 °C. On days 3 and 6 of culturing, fresh medium containing mGM-CSF (200 U/ml) was added. Non-adherent cells were collected on day 9, washed and resuspended at 1×10^6 cells/ml in fully supplemented culture medium for further use. These cells were morphologically, phenotypically (CD11c+ CD11b+ MHC class II+ Gr1- CD4- CD8- CD19-) and functionally (potent APC in allogeneic mixed leucocyte reactions) consistent with DC (data not shown).

A 1 ml cell suspension containing 1×10^6 cells was seeded in 24-well plates and cultured in the presence of 10 µg/ml of either purified triterpenes or the total triterpene fraction. Vehicle (0.5% DMSO) and dexamethasone (3.16 µg/ml, Alexis Biochemicals; also prepared in 0.2% v/v DMSO as vehicle) treatments were used as negative and positive controls, respectively. After a 6 h incubation, cells were stimulated with LPS (0.1 µg/ml, Sigma) for additional 18 h. DC cultures incubated in the absence of LPS were set as the non-stimulated controls. Cell-free supernatants were collected for further assessment of cell viability, cytokine secretion and nitric oxide (NO) production.

Cell viability, cytokine and nitric oxide measurements

DC viability was determined by quantifying the activity of the enzyme lactate dehydrogenase released from the cytosol of DC into the supernatant. For this purpose, a Cytotox 96® Non-radioactive cytotoxicity assay kit (Promega, USA) was used following the manufacturer's protocol. The percentage of viable cells was calculated with the following formula: % viability = 100 – [Experimental LDH release (OD_{490})/Maximum LDH release (OD_{490})]. The concentrations of the cytokines IL-1β, IL-6, IL-12p40, IL-12p70, and TNF-α were determined using Luminex technology and a customised multiplexed kit (Bioplex multiplex mouse cytokine assay kit, Bio-Rad, Hercules, CA), according to the manufacturer's protocol. Data were collected by using the Bio-Plex suspension array system and analysed with the Bio-Plex Manager™ (Bio-Rad, USA) (Lash et al., 2006). The limits of detection of this method for IL-1β, IL-6, IL-12p70, TNF-α, and IL-12p40 were 9.4, 0.2, 2.3, 1.4, and 0.4 pg/ml, respectively. Nitric oxide (NO) production was measured as nitrite equivalents with the Griess reaction in cell-free supernatants as previously reported (Sun J et al., 2003).

Results and discussion

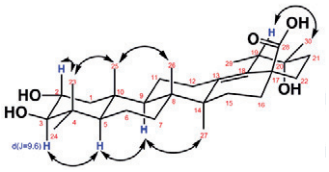
Structural elucidation of yarumic acid (1)

From an extract derived from the roots dissolved in ethyl acetate, an unknown pentacyclic triterpene, which we have defined as yarumic acid (**1**), was isolated and characterised. The exact and unambiguous properties of this compound were made by interpreting the ^1H, ^{13}C, DEPT, COSY, HMQC and HMBC spectra. The stereochemistry of the five ring systems was established by their NOESY and COSY spectra. The elemental composition of $C_{30}H_{48}O_5$ (487.3429) was determined by means of APCI-TOF-MS *m/z* 487.3456 (5.54 ppm error). The ^{13}C-NMR spectra displayed thirty carbon atoms, with one carbonyl at δ 177.46 (qC) and two olefin carbons at δ 139.93 (qC) and 131.34 (qC). By dissimilarity with the DEPT135

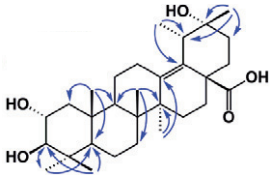
experiment, six quaternary carbons were identified in addition to seven methyl, nine methylene and five methine groups. Three resonances for carbons bearing oxygen were observed at δ 83.54 (qC), 82.99 (CH) and 68.28 (CH). From the HMBC experiment, the double bond was located by CH_3-27 to C-13 and CH_3-29 to C-18 correlations. The relative configuration of the hydroxyl at C-2 was deduced to be α (axial) by strong NOESY correlations at the 2-β-H position with 23-Hs, 23-Hs with 25-Hs and 25-Hs with 26-Hs. Moreover, 3-α-H (doublet) had a coupling constant of δ 9.6, which is suggests to be anti-periplanar to 2-β-H. The 3-α-H position was confirmed by NOESY from 5-α-H, 5-α-H with 9-α-H and 9-α-H with 27-Hs. The 30-β-methyl position was indicated by NOESY from the 19-β-H. Some of the correlations mentioned above are shown in Fig. 1.

Compound **1**: pale yellow amorphous powder; ^{13}C-NMR (100 MHz, CD_3OD δ ppm) see table 1. ^{1}H-NMR (400 MHz, CD_3OD δ ppm) 2.10 (1H, m, H-1b), 0.95 (1H, m, H-1a), 3.65 (1H, m, H-2), 2.94 (1H, d, H-3), 0.88 (1H, m, H-5), 1.60 (2H, m, H-6), 1.51 (2H, m, H-7), 1.62 (1H, m, H-9), 2.05 (1H, m, H-11a), 2.51 (1H, d, H-11b), 1.67 (2H, m, H-12), 1.64 (2H, m, H-15), 2.23 (2H, m, H-16), 2.70 (1H, d, H-19), 1.57 (2H, m, H-21), 1.83 (2H, m, H-22), 1.02 (3H, s, H-23), 0.80 (3H, s, H-24), 0.97 (3H, s, H-25), 0.86 (3H, s, H-26), 1.19 (3H, s, H-27), 1.09 (3H, d, H-29), 1.39 (3H, s, H-30). HRESIMS *m/z* 487.3456 (calcd. for $C_{30}H_{48}O_5$ 487.3429). ESI-IT-MS^2 [M]-*m/z* 487, MS^2: *m/z* 469.2 (100), 453.2 (15), 441.2 (19), 425.2 (12).

NOESY



HMBC



Structural information of known pentacyclic triterpenes

Fig. 1 - Experimental correlations in NOESY and HMBC observed in compound **1**.

Table 1
^{13}C NMR Data of Compounds **1-5**.

Position	Compunds				
	1	**2**	**3**	**4**	**5**
1	47.0	38.0	38.0	38.7	48.0
2	68.1	26.9	23.3	27.4	67.7
3	82.8	76.8	80.9	77.3	82.7
4	38.9	38.3	45.8	38.8	39.4
5	55.2	54.8	55.3	55.2	55.3
6	18.1	18.0	18.1	18.5	18.5
7	34.6	32.4	32.5	33.2	34.6
8	42.9	38.8	39.2	37.0	39.0
9	50.6	47.1	47.5	47.5	47.7
10	38.1	36.6	23.5	37.0	38.0
11	27.3	22.8	27.6	23.3	28.4
12	21.2	121.9	123.2	125.0	125.3
13	139.9	143.5	142.9	138.7	138.9
14	42.3	41.2	41.3	42.1	44.6
15	26.9	27.2	27.6	28.0	23.2
16	24.9	22.7	23.0	24.3	31.2
17	41.2	44.9	37.6	39.8	48.9
18	131.3	42.0	42.2	52.8	133.6
19	39.8	41.6	41.9	38.9	135.0
20	83.5	43.2	43.7	74.3	34.2
21	32.8	29.7	30.3	29.5	26.5
22	32.0	33.2	33.5	36.8	34.9
23	27.7	28.2	28.0	28.7	29.3
24	15.8	16.0	15.3	16.5	17.7
25	16.7	15.1	14.1	15.7	17.5
26	16.8	16.7	16.6	17.4	18.2
27	19.3	25.5	25.8	23.7	22.0
28	177.5	178.1	183.5	178.8	177.4
29	15.5	27.8	28.3	17.5	19.5
30	21.5	176.2	176.9	21.5	19.0
31		51.5	3.6		
32			171.0		
33			21.2		

The structures of four additional oleanane-type triterpenes isolated from *C. telenitida* were established on the basis of spectral analyses and compared to data previously reported (Begum et al., 2002a; Numata A et al., 1990; Razdan et al., 1983). The 13C-NMR data of the isolated pentacyclic triterpenes are shown in Table 1. The molecules were characterised and also traced in the total PT fraction by tandem mass spectrometry. The negative MS spectrum in SIM mode of all of the pentacyclic triterpenes was used to control for the different batches of PT fractions and therefore confirm the presence of more abundant triterpenes (Fig. 2). Following isolation, compounds **1**, **2**, **3**, **4**, and **5**, as well as the total PT fraction, were further evaluated in LPS-stimulated DC.

Immunomodulatory activity of compounds on dendritic cells

DC have been recognised as crucial players during the initiation and maintenance of pathological inflammation largely through their ability to secrete proinflammatory cytokines and trigger a subsequent inflammatory adaptive immune response (Chan et al., 2012; Farkas and Kemény, 2012; Steinman and Hemmi, 2006). Thus, natural products that interfere with proinflammatory pathways in DC are by definition potential promoters of immune regulation and thus possible anti-inflammatory agents (Hackstein and Thomson, 2004; Svajger et al., 2010). An increasing number of reports that assessed the effect of natural products in the form of crude

extracts, fractions or isolated compounds in DC has been reported in recent years (Figueirinha et al., 2010; Freysdottir et al., 2011; Kwon et al., 2011; Liu et al., 2008; Mitsui et al., 2010; Wismar et al., 2011). However, to our knowledge, little information is available on the immunomodulatory capacity of *Cecropia* ssp., and no information regarding either cytokine production or DC modulation by *C. telenitida* metabolites has been reported. Although four of the PT identified in this study have been isolated from other natural sources (Begum et al., 2002b; Dini et al., 2002; Dini et al., 2001; Galarraga et al., 2010; Hänsel and Kußmaul, 1975; Montoya et al., 2009), no effects on proinflammatory cytokine production and DC functions have been assessed. We prepared DC from C57BL/6 mice and determined the ability of PT from *C. telenitida* to modulate cytokine secretion using a multi-analyte luminex assay. Previous experiments demonstrated that none of the isolated compounds or the total PT fraction exerted either a cytotoxic effect or cytokine-inducing activity in resting DC (data not shown). LPS treatment induced prominent increases of IL-1β (2596 +/- 404 pg/ml), IL-12p40 (21013 +/- 4955 pg/ml), IL-12p70 (732 +/- 109 pg/ml), TNF-α (12338 +/- 3108 pg/ml) and IL-6 (7652 +/- 1609 pg/ml) levels. The effect of pre-treatment with either the PT fraction or isolated compounds was determined in LPS-stimulated DC. Cells treated with dexamethasone and vehicle were used as positive and negative control cultures, respectively. At the concentration tested, none of the treatments affected cell viability in LPS-activated DC (Fig. 3). As expected, dexamethasone exhibited a profound anti-inflammatory effect in DC, as evidenced by the almost complete abrogation of LPS-induced IL-1β, IL-12p40, IL12p70 and TNF-α secretion (70-90% inhibition, Fig. 3) and the pronounced inhibition of IL-6 secretion (47%, data not shown). Nitric oxide production was also inhibited by dexamethasone (Fig. 3). All of the PT compounds tested as well as the total PT fraction exhibited anti-inflammatory activity, but the extent of the effects were dependent on the specific compound and cytokine. IL-6 was not modulated by any of the PT treatments (data not shown). Interestingly, the total PT fraction exhibited the most potent modulatory effect, and its inhibitory power was comparable to that of dexamethasone (see the inhibition of IL-1β, IL-12p40, IL12p70 and TNF-α in Fig. 3). Among the isolated compounds, compound **3** was the strongest overall inhibitor of cytokine production (more than 50% inhibition for IL-1β, IL-12p40, IL-12p70 and TNF-α secretion, Fig. 3). Compound **2** was a strong inhibitor of IL-1β and TNF-α (more than 50% inhibition) but a weaker inhibitor of IL-12p40 and IL-12p70 (less than 50% inhibition). The novel compound **1** significantly inhibited IL-1β and TNF-α, but was weak at inhibiting IL-12p40 and IL-12p70. Finally, compounds **4** and **5** were the least active PT, as they significantly inhibited the production of only one of the tested cytokines (IL-12p40 and TNF-α, respectively) by DC (Fig. 3). Regarding NO production, an approximately similar profile of inhibition by PT was observed. Compound **3** was the strongest inhibitor of the production of this mediator, followed by the total PT fraction. Compounds **1**, **2**, **4** and **5** were essentially inactive at the concentration tested (Fig. 3).

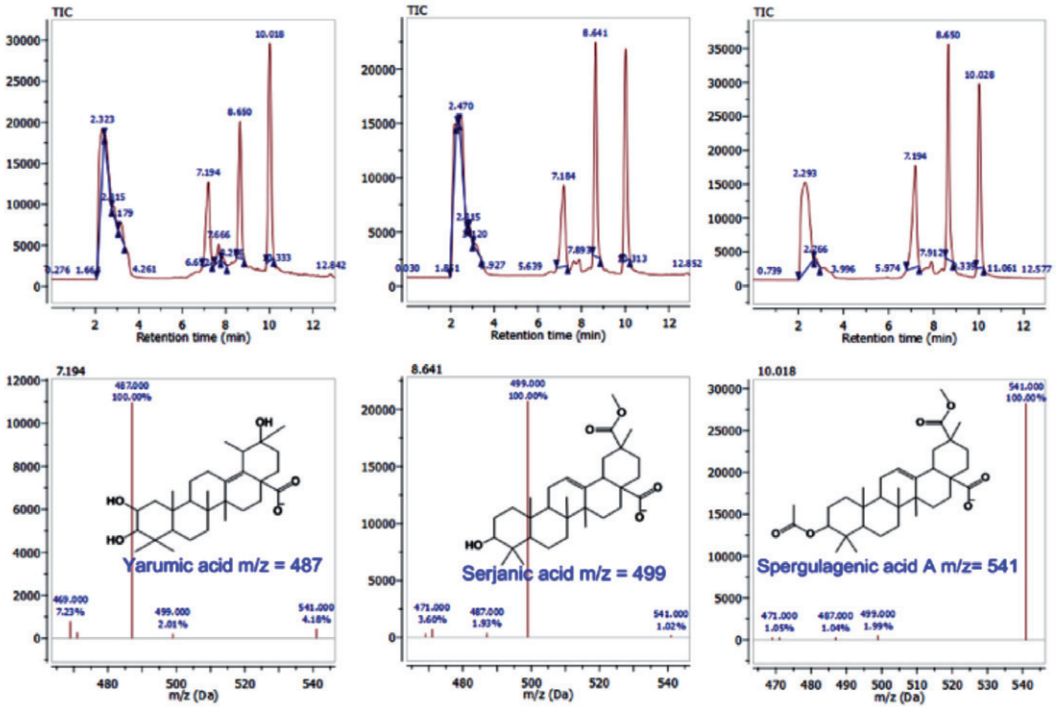


Fig. 2 - SIM spectra in negative mode of different batches of the PT fraction.The retention time shows the mass spectrum of each major component: yarumic acid (1) *m/z* = 487; serjanic acid (2) *m/z*= 499; and spergulagenic acid A (3) *m/z*= 541. The retention times for compounds (4) and (5) are between (1) and (2) (data not shown).

In summary, this investigation resulted in the discovery and identification of **1**, a novel PT of the Oleanane family. Additionally, four known oleanane-type PT were also identified in the roots of *C. telenitida* (serjanic acid, spergulagenic acid A, goreishic acid I and 20-hydroxy-ursolic acid, with the first two being by far the most abundant in the total PT fraction). This is, to our knowledge, the first chemical characterisation of PT reported for this species. Our results are in agreement with previous reports of terpenoids as important active metabolites produced by the *Cecropia* genus (Costa et al., 2011; Schinella et al., 2008). Furthermore, our DC-based assay demonstrated that these PT are powerful inhibitors of proinflammatory cytokine production in this cell type, suggesting the potential of these compounds as immunoregulatory agents. We observed that the total PT fraction from *C. telenitida* exhibited a powerful inhibitory response in inflammatory DC, as assessed by the production of proinflammatory cytokines and NO. This effect was primarily reproduced by compounds **2** and **3**, which were the two most abundant components in the PT fraction (representing the 95% of the total PT mass). The novel PT **1**, which was less representative in the PT fraction (less than 5% of the total PT mass) was also inhibitory, whereas the two minor compounds (**5** and **4**, representing less than 1% of the total PT fraction) exhibited the weakest of bioactivity (Fig. 3). These results suggest that **2** and **3** are responsible for the immunomodulatory activity of the PT fraction from *C. telenitida* in DC. In accordance to our results, a lupane-type triterpenoid from natural origins also inhibited LPS-

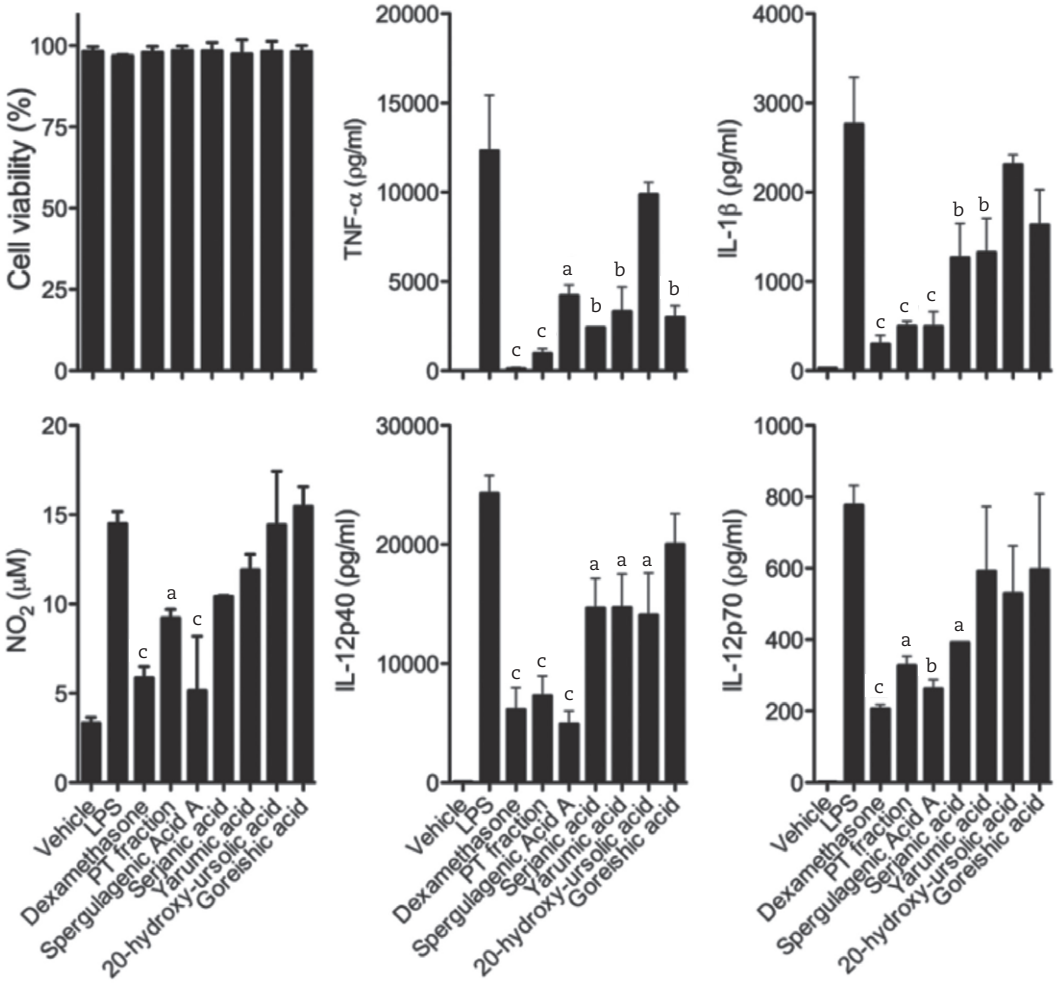


Fig. 3 - Pentacyclic triterpenes from the roots of Cecropia telenitida are immunomodulatory in mouse DC. Cells were generated as described in the experimental section, pretreated with the indicated compound for 6 hours (10 μg/ml), followed by subsequent stimulation with LPS (0.1 μg/ml) for an additional 18 h. Cells treated only with LPS or with LPS plus dexamethasone were used as negative and positive controls, respectively. An additional control of cells treated with vehicle only (DMSO) was also included to confirm the responsiveness of DC to LPS. Supernatants were collected to evaluate cell viability, cytokine secretion and nitric oxide production. The results shown are the mean ± SEM of pooled data from two independent experiments (n = 2 each). Statistical significance of the results was determined by ANOVA using the Dunnett test for multiple comparisons to contrast the cytokine levels secreted by DC receiving multiple treatments to the amount secreted by DC treated with LPS only.[a]$p < 0.05$, [b]$p < 0.01$, [c]$p < 0.001$.

stimulated cytokine production by mouse DC (Kim et al., 2010). Interestingly, some reports have shown that other PT stimulate DC to mature and promote the activation of Th1 and Th2 cells in human (Jung et al., 2010) and mouse (Yu et al., 2009) models, respectively, suggesting structure- and/or species-dependent effects of PT on DC. Opposing effects on proinflammatory mediators (cytokines and NO), depending on the cellular status and environment, have also been reported with several prototypical PT *in vitro* (Choi et al., 2001; Ikeda et al., 2007; Marquez-Martin et al., 2006; Suh et al., 1998; Yun et al., 2003). The *in vivo* implications of these apparently contradictory results remain unclear. Although PT have been shown to affect important signalling pathways involved in the inflammatory response, such as MAPK, NF-κB, Fas, PI3K/Akt and Wnt/β-catenin (Laszczyk, 2009; Poeckel and Werz, 2006; Saleem, 2009); future experiments are required to elucidate the molecular mechanisms mediating the modulatory effect reported here. However, because NF-κB is a major inflammatory signalling pathway triggered after TLR4-mediated activation by LPS in DC, we speculate that this could be a major mediator of PT action in our assays.

Authorship

FA collected plant material and herbarium confections. GLM-P and JAS conducted all laboratory work. JRR-P designed all of the biological experiments carried out. UH interpreted the NMR data and provided lab facilities. All the authors have read the final manuscript and approved the submission.

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