Leishmanicidal and Trypanocidal Activities of Acetogenins Isolated from Annona glauca

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INTRODUCTION

The Annonaceous acetogenins are bioactive natural compounds, they are known to exhibit interesting biological activities including cytotoxic, insecticidal and antiprotozoal activities (Cavé et al., 1997; Fang et al., 1993; Rupprecht et al., 1990). Previous studies on extracts containing acetogenins have described activities against parasites such as Entamoeba histolytica, Trichomonas vaginalis and helminths (Nippostrongylus brasiliensis and Molinema desseatae) (Bories et al., 1991). The leishmanicidal and trypanocidal activities of these compounds have been reported against Leishmania species and the bloodstream forms of Trypanosoma cruzi. Annonacin A and goniolamycin showed activity against Leishmania, and glaucanisin, squamocin, annonacin A and annonacin against Trypanosoma cruzi reducing the parasites by 78%, 67%, 71% and 85%, respectively. © 1998 John Wiley & Sons, Ltd.

Plant material. Seeds of A. glauca were collected in September 1994 in Senegal by D. Fall and authenticated by Professor A. Le Thomas, Museum National d’Histoire Naturelle, Paris.

Extraction and isolation. The dried pulverized seeds (680 g) were macerated with MeOH. The MeOH extract was diluted with 10% volumes of water and submitted to liquid–liquid partition with hexane, leading to 24 g of a concentrated extract. The hydromethanol phase was extracted with CH2Cl2. The concentrated CH2Cl2 extract (14 g), containing acetogenins (Kedde +), was fractionated by flash chromatography on silica-gel 60 and eluted with solvents of increasing polarity leading to several fractions. Two of which were chromatographed over a silica-gel 60 H column and elution by a mixture CH2Cl2–AcOEt–MeOH (90:8:3) affording four acetogenins (see Fig. 1). Glaucanisin (1), rolliniastatin-2 (2) and squamocin (3) were obtained as pure fractions. HPLC was used to verify the purity of them (Fujimoto et al., 1989; Pettit et al., 1989; Waechter et al., 1995). Glaucanillin (4) was

MATERIALS AND METHODS

General experimental procedures. Optical rotations were determined on a Schmidt-Haensch Polartronic I polarimeter. UV spectra were obtained on a Philips PU 8720 spectrometer, IR spectra were measured on a Perkin-Elmer 257 spectrometer. The 1H-NMR and 13C-NMR spectra (CDCl3) were obtained with Bruker AC-200 or AC-400 instruments at 200 and 50 MHz or at 400 and 100 MHz, respectively. EIMS and CIMS (methane) were performed on a Nermag R10-10C spectrometer. HPLC analytic analyses were performed with a Waters 501 pump, a Waters 991 spectrophotometer (214 nm) and a Waters WISP automatic injector on a μBondapak C18 prepacked column (10 μm, 8 × 100 mm), elution with MeOH–H2O at various gradients at a flow rate of 1 mL/min. Preparative HPLC was carried out with a Millipore-Waters (Milford MA, USA) system equipped with a 590 pump, a SSV injector, and a 484 UV detector (214 nm), on a μBondapak C18 prepacked column (10 μm, 25 × 100 mm), elution with MeOH–H2O at various gradients at a flow rate of 10 mL/min.

Keywords: Annona glauca; Annonaceae; acetogenins, biological activities; Leishmania species; Trypanosoma cruzi.
obtained as an almost pure fraction and was further purified by HPLC (Waechter et al., 1997). One fraction, chromatographed equally on a silica-gel 60 H column and elution by a mixture CH₂Cl₂-AcOEt-MeOH (90:8:3) afforded two acetogenins. Molvizarin (5) and parviflorin (6) were further purified by preparative HPLC (Cortes et al., 1991; Ratnayake et al., 1994). Another fraction was chromatographed over a silica-gel 60 H column; elution by a mixture CH₂Cl₂-AcOEt-MeOH (90:8:3) afforded two acetogenins. Molvizarin (5) and parviflorin (6) were further purified by preparative HPLC (Cortes et al., 1991; Ratnayake et al., 1994). Another fraction was chromatographed over a silica-gel 60 H column; elution by a mixture CH₂Cl₂-AcOEt-MeOH (80:15:5) afforded two acetogenins. Annonacin A (7) and annonacin (8) were obtained as pure fractions proved by HPLC (Lieb et al., 1990; McCloud et al., 1987). The last studied fraction was chromatographed over a silica-gel 60 H column and elution by a mixture CH₂Cl₂-AcOEt-MeOH (80:13:7) afforded gonoiothalamacin (9) as a pure fraction (HPLC) (Alkofahi et al., 1988). Compounds 1-9 showed identical spectral data to those previously reported for these compounds.

Leishmanicidal activity. Cultures of Leishmania spp were obtained from IICS (Instituto de Investigaciones en Ciencias de la Salud, Asuncion) and identified by isoenzyme analysis. Three strains of Leishmania were used during these investigations: L. braziliensis (MHOM/BR/75/M2903), L. amazonensis (IFLA/BR/67/PH8) and L. donovani (MHOM/IN/83/HS-70) grown at 22°C in Schneider’s Drosophila medium containing 20% fetal bovine serum. Compounds were dissolved in 5 µL of dimethyl sulphoxide (DMSO), then in medium and placed in microtite plates in triplicate. The minimal amount (µg) of compound to inhibit the growth of Leishmania sp was evaluated after 48 h by optical observation on a drop of each cell culture with a microscope by comparison with control cells and with reference drugs (N-methylglucamine antimonate and pentamidine). The maintenance, cultivation and isolation of promastigote-stage parasites have been described in detail elsewhere (Fournet et al., 1994). Pentamidine was used as a positive control (Fournet et al., 1993).

Trypanocidal activity. Albino mice infected with Trypanosoma cruzi strain, 7 days after infection were used. Blood was obtained by cardiac puncture using 3.8% sodium citrate as anticoagulant in a 7:3 blood/anticoagulant ratio. The parasitaemia in infected mice ranged between 1×10⁵ to 5×10⁵ parasites per millilitre. Plant extracts and isolated compounds were dissolved in cold DMSO to a final concentration of 250 µg/mL. Aliquots of 10 µL of each extract of different concentrations (4, 20, 40, 100 and 250 µg/mL) were mixed in microtite plates

((Continued from the previous page))

Scheme 1. Acetogenins from Annona glauca.

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with 100 μL of infected blood containing different parasite concentrations (1 × 10⁵ and 10⁶ parasites per mL). Infected blood and infected blood containing gentian violet at 250 μg/mL were used as controls. The plates were shaken for 10 min at room temperature and kept at 4°C for 24 h. Each solution was microscopically observed at 400x, placing a 5 μL-sample on a slide and covering it with a 22 × 22 mm coverslip for parasite counting (Rojas de Arias et al., 1994; Schempler, 1978).

**RESULTS AND DISCUSSION**

The hexane extract of *A. glauca* did not present a significant activity against *Leishmania* promastigotes, whereas the dichloromethane extract had an IC₅₀ value of 25 μg/mL (Table 1). The same activity was found with the isolated compounds: glaucanisin (1), rolliniastatin-2 (2), squamocin (3), glaucafilin (4) and annonacin (8). Molvizarin (5), a C₃₅ acetogenin and its isomer, parviflorin (6), did not show activity. Annonacin A (7) and goniothalaminic (9) were quite active towards *Leishmania* promastigotes, showing IC₅₀ values of 10 and 5 μg/mL respectively.

Some structure–activity relationship trends of these compounds can be considered. The width of the alkyl chain of the bis-THF acetogenins (compounds 1, 2, 3, 5 and 6) seems to influence the weak leishmanicidal activity of these compounds. In effect, molvizarin (5) and parviflorin (6), two C₃₅ bis-THF acetogenins, were inactive against *Leishmania* spp, but glaucanisin (1), rolliniastatin-2 (2) and squamocin (3), C₃₇ bis-THF acetogenins, presented an IC₅₀ value of 25 μg/mL. Neither the sub-type of lactone (1a or 1b) nor the number of hydroxy groups seems to be implicated in the activity of these molecules (see Fig. 1) (Cavé et al., 1997).

The compounds 4, 7, 8 and 9 are C₃₅ mono-THF acetogenins (sub-group 1b) with four hydroxy groups, these compounds presented IC₅₀ values of 25, 10, 25 and 5 μg/mL, respectively against *Leishmania* spp. The difference in activity between 8 and 9 and on the other hand between 7 and 8 is probably due to the position of THF-ring and its relative configuration. Considering these results, it is not easy to establish a structure–activity relationship, nevertheless the mono-THF acetogenins seem to be more leishmanicidal than the bis-THF acetogenins.

The hexane extract from *A. glauca* did not present an activity against the bloodstream forms of *Trypanosoma cruzi*, but some acetogenins, glaucanisin (1), squamocin (3), annonacin A (7) and annonacin (8) produced a reduction of the parasites of 78%, 67%, 71% and 85% (Table 2). This level of activity is considered as

**Table 2. In vitro activity of *A. glauca* crude extracts and acetogenins on bloodstream forms of *Trypanosoma cruzi* (IC₅₀ μg/mL)**

<table>
<thead>
<tr>
<th>Extract or compound</th>
<th><em>T. cruzi</em> (UC4)</th>
<th><em>T. cruzi</em> (IHS-70)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hexane extract</td>
<td>&gt;100</td>
<td>&gt;100</td>
</tr>
<tr>
<td>Glaucanisin (1)</td>
<td>78</td>
<td>78</td>
</tr>
<tr>
<td>Rolliniastatin-2 (2)</td>
<td>13</td>
<td>13</td>
</tr>
<tr>
<td>Squamocin (3)</td>
<td>67</td>
<td>67</td>
</tr>
<tr>
<td>Glaucafilin (4)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Molvizarin (5)</td>
<td>41</td>
<td>41</td>
</tr>
<tr>
<td>Parviflorin (6)</td>
<td>35</td>
<td>35</td>
</tr>
<tr>
<td>Annonacin A (7)</td>
<td>71</td>
<td>71</td>
</tr>
<tr>
<td>Annonacin (8)</td>
<td>85</td>
<td>85</td>
</tr>
<tr>
<td>Goniothalaminic (9)</td>
<td>32</td>
<td>32</td>
</tr>
<tr>
<td>Gentian violet</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

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significant (Croft et al., 1988). Alone, annonacin A (7) proved to be active in the bioassay against *Leishmania* spp. and *T. cruzi*. The dichloromethane extract showed high cytotoxicity (IC$_{100}$ 5 × 10^{-5} µg/mL) and the hexane extract a weak toxicity (IC$_{100}$ 1.5–5 µg/mL) against KB and Vero cell lines (Table 3). These results are coherent, since the dichloromethane extract contained the acetogenins 1–9.

In conclusion, the results obtained in this study suggest that it would be interesting to continue the biological investigations of some acetogenins against *Trypanosoma cruzi*, using an animal or cellular model. More experiments, especially with a larger number of compounds (position and configuration isomers) may be helpful to elucidate precise structure–activity relationships.

**REFERENCES**


