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Antiprotozoal and antiangiogenic saponins from *Apodytes dimidiata*

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ABSTRACT

Bioassay-guided isolation was performed on the leaves of *Apodytes dimidiata* E. Mey. Ex Arn. (Icacinaceae), based on previously demonstrated activity against *Leishmania*. Six saponins never isolated from nature before were elucidated with LC–MS/MS, GC–MS and 1D and 2D NMR. The compounds apodytine A–F are responsible at least in part for the antiprotozoal activity, but also possess haemolytic activity and display antiangiogenic activity in the rat aorta ring assay, an effect which may be due to a non-selective toxicity.

1. Introduction

The plant *Apodytes dimidiata* E. Mey. Ex Arn. (Icacinaceae) (Sleumer, 1971), also known as white pear, is particularly common not only in southern parts of Africa, but also in eastern Africa and different Asian countries. Traditionally the bark and leaves are used as a purgative for calves, to treat worms in cattle, and a decoction is used as enema for intestinal parasites (Gestner, 1938; Bryant, 1966; Hutchings et al., 1996). The leaf is used in the treatment of ear inflammation (Watt and Breyer-Brandwijk, 1962).

Several extracts of different parts of the plant displayed a pronounced activity against the snails *Bulinus africanus* and *Biomphalaria pfeifferi*, the intermediate host snails of *Schistosoma* spp. While an aqueous extract of the leaves of the plant was found to be extremely toxic, causing 100% mortality of both snail species within 24 h at concentrations of 100 mg/l, the extract of the bark, roots and fruits displayed only mild molluscicidal effects (Pretorius et al., 1991). Since the control of schistosomiasis with synthetic molluscicides is beyond the economic reach of developing countries and may be hazardous because of inappropriate use, plant molluscicides might offer an alternative (Brackenbury, 1999). Since *A. dimidiata* displayed activity against snails and was classified as non-toxic and non-irritating based on acute and sub-acute mammal toxicity tests, this plant may be useful in the fight against schistosomiasis (Brackenbury et al., 1997a).

The molluscicidal activity of the bark was attributed to the iridoid genipin and its 10-acetyl derivative. Both compounds displayed molluscicidal activity with a LD_{90} of 32 ppm and 39 ppm (Drewes and Kayonga, 1996), being a rather moderate activity. However, there were indications that not a single compound was responsible for all the molluscicidal activity, but that also saponins may be present, as indicated by the foam formed during extraction, the haemolysis that occurred when snails made contact with the extract, and the piscicidal activity (Brackenbury and Appleton, 1997b; Brackenbury, 1999). Previous studies also revealed *in vitro* inhibition of *Leishmania* of a methanol extract of the leaves of *A. dimidiata* (Maes, 2003). Since plants are still important sources of new lead compounds, a bioassay-guided isolation was carried out to isolate and identify the active principles. Six saponins responsible at least in part for the observed antileishmanial activity were isolated of which the antiprotozoal, haemolytic and antiangiogenic activity was investigated.

2. Results and discussion

2.1. Structure elucidation

The crude methanol (80%) extract of the leaves of *A. dimidiata* was extracted using subsequently petroleum ether, dichloromethane and *n*-butanol saturated with H_2O. Column chromatography

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Table 1
Chemical formulas and accurate mass measurements of the deprotonated molecules $[M-H]^-$, isolated from fraction AD I and the optical rotation of the compounds.

Compounds	Chemical formula $[M-H]^-$	$[M-H]^-_{calculated}$	$[M-H]^-_{experimental}$	Error (ppm)	$[\alpha]^{20}$
1	$C_{60}H_{93}O_{28}$	1261.5848	1261.5845	−0.252	+26 (c 0.21, DMSO)
2	$C_{59}H_{91}O_{27}$	1231.5742	1231.5764	1.670	+25 (c 0.16, DMSO)
3	$C_{60}H_{95}O_{27}$	1247.6055	1247.6048	−0.564	+15 (c 0.20, DMSO)
4	$C_{60}H_{93}O_{28}$	1261.5848	1261.5849	0.089	+16 (c 0.26, DMSO)
5	$C_{59}H_{91}O_{27}$	1231.5742	1231.5741	−0.076	[a]
6	$C_{60}H_{95}O_{27}$	1247.6055	1247.6047	−0.660	+17 (c 0.16, DMSO)

[a] Optical rotation could not be determined.

was performed on the *n*-BuOH fraction. One of the obtained fractions was subjected to semi-preparative HPLC and resulted in the isolation of three fractions (AD I, AD II and AD III). Semi-preparative HPLC on the first fraction resulted in the isolation of 6 saponins of which the *m/z* values of the deprotonated molecules $[M-H]^-$ compounds **1–6** and the optical rotation are shown in Table 1. Information of the structure of the compounds present in fraction AD I was obtained by the analysis of the MS^n data. Fig. 1 and Table 2 show the MS^2 data obtained for the 1232 MW compound **5**. The *m/z* 1231 MS^2 product ion spectrum reveals ions at *m/z* 1069 and 1051, corresponding to Y and Z type ions formed by the loss of an hexose residue (162 u) and a hexose (180 u), respectively, and an ion at *m/z* 1099, corresponding to the Y ion formed by the loss of a pentose residue. Both hexose and pentose are found in a terminal position of the molecule. Other product ions generated from *m/z* 1231 included *m/z* 967 $[M-H-264]^-$, *m/z* 787 $[M-H-444]^-$ and *m/z* 629 $[M-H-602]^-$. The formation of product ion *m/z* 967 $[M-H-264]^-$ can be attributed to the loss of two pentose residues, product ion *m/z* 787 $[M-H-444]^-$ to the loss of two pentose residues and one hexose residue and product ion *m/z* 629 $[M-H-602]^-$ is generated by the loss of two pentose residues, one hexose residue and one hexuronic acid, respectively. The ion at *m/z* 1129 $[M-H-102]^-$ in the *m/z* 1231 MS^2 product ion spectrum can be explained by the loss of a pentanoic acid residue. Several important MS^2 product ions $[M-H]^-$ of the other compounds present in fraction AD I are listed in Table 2. The fragmentation

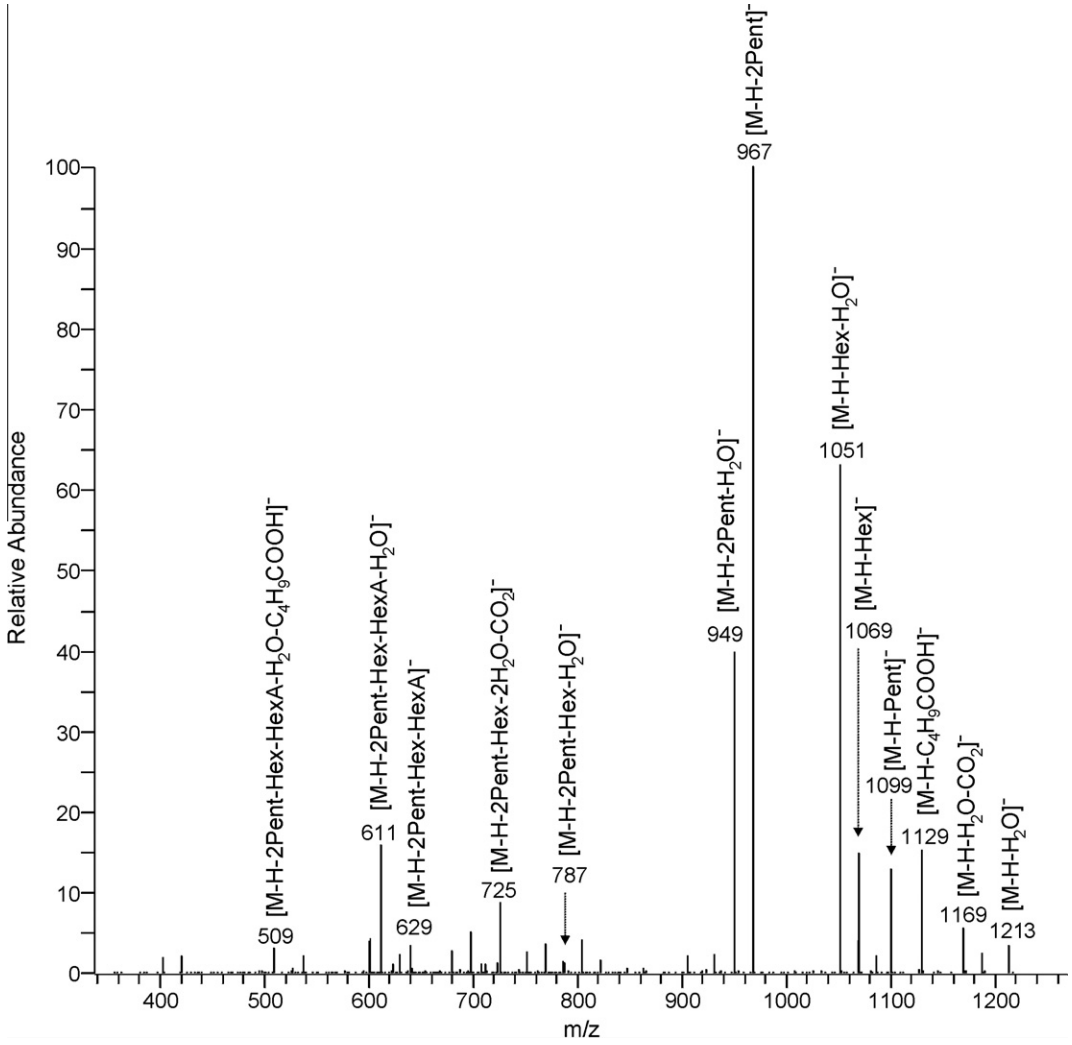

Fig. 1. MS^2 spectrum of the product ion *m/z* 1231 of compound **5**.