Iridoid Molluscicidal Compounds from *Apodytes dimidiata*

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The bark of *Apodytes dimidiata* (Icacinaceae) yielded two major compounds, the iridoid genipin (**1**) and its 10-monoacetate derivative **2**. Using *Bulinus africanus* as host snail, we show here that LD_{50} values for both compounds were in the range 22 −26 ppm.

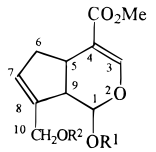
Much has been written about plant molluscicides, both from the point of view of using pure synthetic chemicals as active agent, or alternatively, relying on relatively crude extracts from selected plants.[1−3] In particular, the studies of Lemma in Ethiopia on *Phytolacca dodecandra* ("ENDOD") have received widespread attention.[4] Despite many years of research on this plant, the process has not been officially endorsed for wide-scale application in Third World countries by the World Health Organization (WHO).

Aqueous suspensions of plant material are a practical form of molluscicide control in rural areas of Southern Africa where bilharzia (schistosomiasis) today poses a real threat to health. With these considerations in mind, a survey of plant molluscicide sources was undertaken by one of us (T.E.C.) using the information available from the WHO on this subject.[5] The result of this selection procedure[6] identified *Apodytes dimidiata* E. Meyer ex Arn. (Icacinaceae) as one of three local indigenous plants with potential as a molluscicide. Previously Joubert and co-workers[7] had evaluated extracts from the leaves, berries, and bark of this tree, but no attempt was made to isolate or identify the active agent.

Identification of the major component in the bark as genipin (**1**) was confirmed by reference to the work of Djerassi[8,9] on the isolation of genipin from the ripe fruits of *Genipa americana* (Rubiaceae). In Djerassi's paper,[9] only proton chemical shift values at 60 MHz were quoted for **1**. Because ^{13}C-NMR data are not available in the literature for this compound, they are given in this paper.

Genipin 10-acetate (**2**) has not been isolated previously from natural sources. There are only two previous references in the literature to the compound. Takeda and co-workers[10] report it as an intermediate in the hydrolysis of the naturally occurring 10-acetylgeniposide, and Isoe[11] quotes it as an intermediate product in his synthesis of the unusual iridoid cerbinal.

The identification of **2** was accomplished by comparison of the ^{1}H- and ^{13}C-NMR data for this compound with our 200 MHz spectral data for genipin (**1**) and also with the aid of the partial ^{1}H-NMR information quoted in Takeda's paper[10] for 10-acetyl genipin (**2**). Location of the acetyl group at C-10 was based on the observation that the methylene protons attached to C-10 in **1** are shifted downfield from δ 4.28 to δ 4.82 in **2**, whereas the methine proton on C-1 has the same chemical shift in both **1** and **2**. This positively pinpoints the position of attachment of the acetyl group. All other proton shifts could be assigned by direct domparison with data for **1**.



1 Genipin $R^1 = R^2 = H$
2 Genipin acetate
$R^1 = H$
$R^2 = Ac$

A wide range of biological activities has been ascribed to genipin (**1**) and its glucosylated methyl ester. These activities include hepatotoxic effects in rats,[1,2] inhibition of growth in wheat embryos,[13] and anti-tumor-promoting properties.[14] Other than the reference to *A. dimidiata* leaf extracts having molluscicidal activity,[7] there is no mention in the literature of genipin (**1**) behaving as a molluscicide.

In assessing the molluscicidal activity of the components present in *A. dimidiata*, the protocols described elsewhere[6,15] were employed. For this study *Bulinus africanus*, the intermediate host of the parasite *Schistosoma haematobium*, was used as host snail. The most active fraction of the plant material (established by way of preliminary LD_{50} values) was a CH_2Cl_2 extract of the bark from which **1** and **2** were isolated. The LD_{50} and LD_{90} values for **1** and **2** were calculated using Fieller's method as implemented in the GENSTAT 5 program.[16] Actual values, determined with 95% confidence limits, afforded the following results: genipin (**1**), $LD_{50} = 25.27$ ppm (±1.77) and $LD_{90} = 32.57$ ppm (±2.25); genipin 10-acetate (**2**), $LD_{50} = 21.72$ ppm (±6.89) and $LD_{90} = 39.40$ ppm (±14.85). Other components in the bark (unidentified at present) showed decreased activity and are still under investigation.

These toxicity values are reasonable without being spectacular. Studies by one of us (T.B., unpublished) also revealed that toxicity effects of *A. dimidiata* extracts on earthworms, fish, and small mammals

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Table 1. ^{1}H-NMR Data for **2** and ^{13}C-NMR Data for **1** and **2**

	compound **1**	compound **2**	
atom	^{13}C	^{1}H	^{13}C
1	96.3, d	4.79, d (J = 8.6 Hz	96.0
3	152.5, d	7.53, s	152.8, d
4	110.7, s		109.8, s
5	36.7, d	3.19, m	35.7, d
6	39.0, t	(a) 2.05, m	38.3, t
		(b) 2.91, m	
7	131.0, d	5.95, br s	129.0, d
8	141.9, s		138.9, s
9	48.1, d	2.52, m	46.9, d
CO_2*Me*	51.4, q	3.77, s	51.0, q
CH_2OH	61.3, t		
CH_2OAc		4.82 (dd, J = 12.9 Hz)	62.2, t
CO_2Me	168, s		167.1, s
O*Ac*		2.11, s	20.7, q

(rabbits) were extremely low, which is a necessary requirement for any commercial molluscicide.

Experimental Section

General Experimental Procedures. ^{1}H-NMR and ^{13}C-NMR spectra were recorded at 200 MHz and 50 MHz, respectively, with $CDCl_3$ as internal standard. Separations were effected on Si gel 60 columns (medium-pressure flash chromatography) or by the use of centrifugal chromatography [circular plates coated (4 mm) with Merck Kieselgel 60 PF_{254}].

Plant Material. *A. dimidiata* (Icacinaceae) stem bark was collected from trees in the Hayfields area, Pietermaritzburg, South Africa. Voucher specimens (verified by one of us, T.E.C.) were deposited in the Herbarium of the University of Natal, Pietermaritzburg, South Africa (voucher number T.E.C.5−1994). A specimen of stem bark was also obtained from Zimbabwe and identified by Mr. S. Mavi. A voucher specimen (no. 1907) was lodged in the National Herbarium, Harare, Zimbabwe.

Isolation. Milled bark (2.5 kg) was successively extracted at room temperature with hexane, CH_2Cl_2, EtOAc, and EtOH. The CH_2Cl_2 fraction (78.5 g) was filtered through a short column of Si gel (MeOH), and a portion of this material (1.38 g) was separated by column chromatography. At this stage Si gel TLC [EtOAc−hexane, 3:2)] showed the presence of eight components of which the two at R_f 0.62 and R_f 0.26 were major components. Separation with EtOAc−hexane afforded genipin (**1**) (140 mg) as colorless crystals (MeOH); mp 120−121 °C; [α] +109 °C (*c* 0.06, $CHCl_3$); EIMS (70eV) *m*/*z* $[M]^+$ 226 (14), 208 (27), 190 (22), 180 (48), 176 (77), 162 (49), 148 (100), 120 (73), 91 (49), 78 (47); HRMS $[M]^+$ 226.0836, calcd for $C_{11}H_{14}O_5$ 226.0841.

Repeated column chromatographic separation of the higher R_f fraction gave 10-acetyl genipin (**2**) (58 mg) as a colorless oil; $[\alpha]^{20}_D$ +22.6 °C (*c* 0.06, $CDCl_3$) (lit.[10] +48.2 °C) EIMS (70eV) *m*/*z* $[M]^+$ 268 (1.0), 155 (2), 127 (4), 99 (13), 85 (45); HRMS $[M]^+$ 268.0947, calcd for $C_{13}H_{16}O_6$ 268.0939. ^{1}H- and ^{13}C-NMR spectra ($CDCl_3$ as solvent) are shown in Table 1.

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