

An Acetylated Eudesmane Glucoside from *Apodytes dimidiata* Growing in Madagascar

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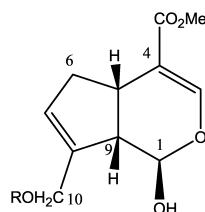
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A new eudesmane glucoside has been isolated from the aerial part of *Apodytes dimidiata*, an Icacinaceae plant widely used in the Southern African countries for its molluscicidal activities. The structure of the new compound was determined by spectroscopic methods. The chemosystematics of the genus belonging to the family Icacinaceae is discussed.

Key words: Icacinaceae, *Apodytes dimidiata*, Eudesmane Glucoside

Introduction

Apodytes dimidiata is renowned in South Africa for its molluscicidal activity. Clark and Appleton have demonstrated that 4 g/l of the crude suspension of the plant collected in South Africa produced 100% mortality of *Bulimus africanus* (the intermediate snail host of *Schistosoma haematobium*-urinary bilharzia), while Pretorius and coworkers recorded 100% mortality at 0.1 g/l [1,2]. Brackenbury and his group investigated the mammal toxicity assessment of the plant and have shown that *A. dimidiata* can be classified as non-toxic and non-irritating according to the European Economic Community (EEC), South African Bureau of Standards (SABS), and the World Health Organization (WHO) [3,4]. The two major constituents of the dichloromethane extract of the South African *A. dimidiata* (bark) were genipin (**1**) and its 10-monoacetate derivative (**2**, Fig. 1). The two compounds show a reasonable molluscicidal activity: (**1**):



1: Genipin: R=H
2: Genipin acetate: R=Ac

Fig. 1. Structure of compounds **1** and **2**.

LD₅₀ = 25.27 ppm (±1.77) and LD₉₀ = 32.57 ppm (±2.25), (**2**): LD₅₀ = 21.72 ppm (±6.89) and LD₉₀ = 39.40 ppm (±14.85) [5]. The decoction of the plant is used in Madagascar Island as molluscicide and anti-inflammatory. No phytochemical studies on *A. dimidiata* collected from Madagascar have been reported so far.

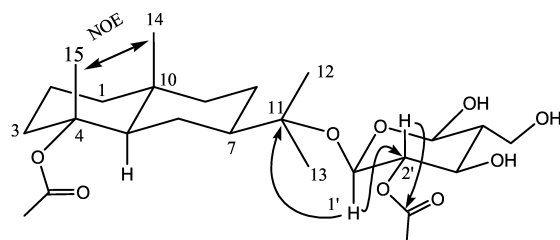
Results and Discussion

A combination of column chromatography on highly porous divinylbenzene, silica gel and reversed phase (RP-18) of the hot methanol extract of *A. dimidiata* led to the isolation of an acetylated eudesmane sesquiterpene glucoside (**3**).

The molecular formula of compound **3** (amorphous powder, $[\alpha]_D^{25} - 36^\circ$, $c = 1.2$ MeOH) was determined as C₂₅H₄₂O₉ by negative HR-FABMS ($[M-H]^-$ 485.2674, requires 485.2751). Inspection of the ¹H NMR spectrum exhibited an anomeric signal ascribable to a β-glucopyranosyl (δ = 4.76, d, $J = 7.8$ Hz), two acetyl groups at δ = 1.99 and δ = 2.01, and four quaternary methyl groups (δ = 0.86, 1.07, 1.15 and δ = 1.23, each singlet). The ¹³C NMR spectral data showed the presence of 25 signals. Six of them could be assigned as belonging to the β-glucopyranosyl unit, four to two acetyl groups and the remaining fifteen to an eudesmane-type sesquiterpene aglycone. The ¹H and the ¹³C NMR data of the aglycone indicated that the planar structure of **3** is very similar to proximadiol (or cryptomeridiol) (**4**, Table 1) previously isolated from an Egyptian Gramineous plant, *Cymbopogon proximus*, except for the presence of the additional signals of one set of β-glucopyranosyl unit and two acetyl groups [6]. The allocation of the functional groups, as depicted, was achieved by intensive 2D-NMR studies. HMB correlations between H-1' (δ = 4.76)/C-11 (δ = 81.8), H-1'/C-2' (δ = 77.3) and H-2' (δ = 4.82)/C=O (δ = 171.3) were observed, in-

Table 1. ^1H (400 MHz) and ^{13}C NMR (100 MHz) data for compounds **3** and **4**.

	^1H	3 *	^{13}C	4 **
1			42.3 (t)	41.1 (t)
2			21.1 (t)	20.2 (t)
3			44.1 (t)	43.6 (t)
4			73.1 (s)	72.3 (s)
5	1.22 dd, $J = 11.5, 4.0$ Hz, H-5		55.2 (d)	54.9
6	(d)			
7			22.2 (t)	21.5 (t)
8	2.12 m		49.5 (d)	50.7 (d)
9			24.0 (t)	22.5 (t)
	1.72 brd, $J = 9.8$ Hz		46.1 (t)	44.7 (t)
	1.30 overlapped			
10			35.6 (s)	34.6 (s)
11			81.8 (s)	73.0 (s)
12	1.23 s		23.8 (q)	27.2 ^a (q)
13	1.15 s		25.5 (s)	27.4 ^a (q)
14	0.86 s		19.2 (q)	18.7 (q)
15	1.07 s		22.5 (q)	22.7 (q)
1'	4.76 d, $J = 7.8$ Hz		96.1 (d)	
2'	4.82 dd, $J = 7.8, 8.3$ Hz		77.3 (d)	
3'	3.70 t, $J = 8.5$ Hz		73.7 (d)	
4'	3.76 t, $J = 8.5$ Hz		70.0 (d)	
5'	3.59 ddd, $J = 8.5, 3.5, 2.3$ Hz		77.7 (d)	
6'	3.78 dd, $J = 12.0, 3.5$ Hz		62.5 (t)	
AcC=O	3.82 dd, $J = 12.0, 2.3$ Hz			
AcC=O	1.99 s		20.8 (q)	
AcC=O	2.01 s		20.9 (q)	
AcC=O			171.3 (s)	
AcC=O			172.2 (s)	

* In CDCl_3 ; ** in CD_3OD .Fig. 2. Important HMBC and NOE correlations observed in compound **3**.

dicating that the β -glucopyranosyl unit was acetylated at the C-2' position and that C-11 was the site of glucosylation. These evidences also showed that the other acetyl group had to be attached at C-4 hydroxyl group. The axial orientation of the methyl at C-4 and the proton at C-7 was deduced from the observation of NOE correlations between CH_3 -14 and CH_3 -15, as shown in Fig. 2. The equatorial orientation of the substituent at C-7 was deduced from the observation of the lower fielded chemical shift of C-9 ($\delta = 46.1$) [6]. Thus the relative configuration of **3** was deduced as shown.

On the basis of the phytochemical data, Kaplan and coworkers suggested a classification of 10 genera of Icacinaceae plants; Group I, for the genera, which contain carbocyclic iridoids: *Apodytes* from Queensland, *Cantleya* of Sumatra, Malaysia and Borneo and *Lasianthera* from New Caledonia; Group II, for those containing alkaloids: *Merriliodendron* of Malaysia, Melanesia and Micronesia, *Nothapodytes* from India and *Cassinopsis* from South Africa; Group III, for the genera, which produce eudesmane and rearranged eudesmane sesquiterpene as emmotins: *Poraqueiba* and *Emmotum* from South America; Group IV for those, which contain diterpenoid derivatives: *Icacina* from tropical Africa and *Humirianthera* from tropical South America (Amazonia) [7]. Since all of the plants investigated produce secologanin or secologanin derived compounds, these iridoid-type compounds might be used as chemotaxonomical marker of the family Icacinaceae. Moreover, we have isolated a new secoiridoid glucoside from *Cassinopsis chapieri* [8]. The detection of carbocyclic iridoid derivatives in *Apodytes dimidiata* from Queensland classified this plant in the first group [9]. This fact was confirmed by the isolation of genipin and its derivative from *A. dimidiata* from South Africa [5]. However, the presence of sesquiterpene in *A. dimidiata* has not yet been reported. The new acetylated eudesmane sesquiterpenoid glucoside (**3**) isolated from the species collected in Madagascar may serve as characteristic feature to distinguish between *A. dimidiata* growing in different geographic localities. *A. dimidiata* might be the link between Group I and Group III since it contains carbocyclic iridoids and eudesmane type sesquiterpenes. Further study needs to be undertaken to determine whether the plants belonging to the two groups have the same chemotypes.

Experimental Section

Plant material

The leaves of *apodytes dimidiata* E. Mey. Ex Arn. (Icacinaceae) was collected in March 2001 near Andasibe (about 140 km from Antananarivo, MADAGASCAR) and deposited in the Herbarium of the Laboratoire de Microbiologie de l'Environnement du Centre National de Recherche sur l'Environnement, Tsimbazaza, Antananarivo MADAGASCAR.

Extraction and isolation

The dried leaves (360 g) of *Apodytes dimidiata* were extracted with hot MeOH. After removal of the solvent by evaporation, the residue (53 g) was suspended in water and ex-

tracted with hexane and EtOAc successively. The aqueous layer (33 g) was subjected to a column of highly porous copolymer of styrene and divinylbenzene, and eluted with H₂O, MeOH and Me₂CO successively. The fraction eluted with MeOH was chromatographed on a column of silica gel (CH₂Cl₂ : MeOH : H₂O, 17 : 6 : 1), affording seven fractions. Fraction 1 was subjected to a column on RP-18 using aque-

ous MeOH gradient (20 % to 100 % MeOH) to afford compound **3** (14 mg).

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