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A MOLLUSCICIDAL AND ANTIFUNGAL TRITERPENOID SAPONIN FROM THE ROOTS OF *CLERODENDRUM WILDII*

MASAO TOYOTA, JEROME D. MSONTHI* and KURT HOSTETTMANN

Institut de Pharmacognosie et Phytochimie, Ecole de Pharmacie, Université de Lausanne, 2 rue Vuillermet, CH-1005 Lausanne, Switzerland; *Chemistry Department, Chancellor College, University of Malawi, P.O. Box 280, Zomba, Malawi

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Key Word Index—*Clerodendrum wildii*; Verbenaceae; roots; molluscicidal and antifungal activity; bitter principle; triterpenoid saponin.

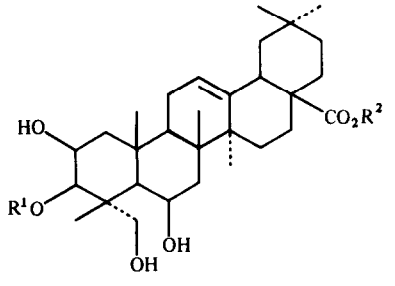
Abstract—From the methanolic extract of roots of *Clerodendrum wildii*, a molluscicidal and antifungal triterpenoid saponin has been isolated and identified as Mi-saponin A by spectral analysis and chemical transformations.

INTRODUCTION

As part of our investigation into the molluscicidal and antifungal properties of African medicinal plants, it was found that the bitter principle from the methanol extract of roots of *Clerodendrum wildii* Moldenke showed activity in both bioassays. Other species of the genus *Clerodendrum* are used in traditional medicine as antimalarials and against intestinal parasites [1]. Phytochemical investigations of this genus have led to the isolation of iridoids [2], flavones, neolignans [3] and caffeic acid glycosides [4, 5]. A bitter diterpene, clerodine, isolated from *Clerodendrum infortunatum* is piscicidal [6], and an antifungal hydroquinone diterpene, uncinatone, isolated from *C. uncinatum* is a rice weevil feeding inhibitor [7, 8]. Triterpenoids have been found in an acidic hydrolysate of a saponin fraction of *C. serratum* [9]. However, no reports on the isolation of saponins of this genus have appeared.

RESULTS AND DISCUSSION

Roots of *C. wildii* were extracted with dichloromethane and then with methanol. The Liebermann–Burchard reaction of the methanol extract showed the presence of saponins and the extract was separated by Sephadex LH-20 into three fractions. Rechromatography on silica gel of the first fraction led to the isolation of a pure bitter compound **1**. The negative-FAB mass spectrum of **1** gave a quasimolecular ion at *m/z* 1221. The ^{13}C NMR spectrum of **1** showed five anomeric carbons (at δ93.2, 101.1, 102.5, 105.5 and 106.6) and one carbonyl carbon (at δ176.3). Acidic hydrolysis of **1** afforded glucose, arabinose, rhamnose and xylose, identified by TLC, the aglycone **2** and an artefact aglycone **3**, identified as protobassic and bassic acid [10] respectively by ^{1}H and ^{13}C NMR. The structure of prosapogenin **4**, obtained by basic hydrolysis of **1**, was confirmed as Mi-glycoside I [11] from ^{1}H and ^{13}C NMR spectral data. Finally, ^{13}C NMR spectral data of **1** suggested that the structure of the oligosaccharide moiety was the same as that of Mi-saponin A. The identity of **1** was confirmed as Mi-saponin A by TLC and HPLC with an authentic sample. Assignment of ^{13}C NMR data for compound **1** (Mi-saponin A) and its derivatives are shown in Table 1, as there is no report of these data in the literature. Bitter saponin **1** (Mi-saponin A) showed molluscicidal activity (25 ppm) against *Biomphalaria glabrata* snails [12] and **1** (< 1.5 μg) and **2** (< 3.3 μg) inhibited *Cladosporium cucumerinum* spore formation in a TLC bioassay [13]. While Mi-saponin A has been isolated previously from *Madhuca longifolia* (Sapotaceae), this is the first report of a saponin from the genus *Clerodendrum* (Verbenaceae).



1 R^1 = Glc, R^2 = Ara(2→1)Rha(4→1)Xyl(3→1)Rha
2 R^1 = R^2 = H
4 R^1 = Glc, R^2 = H

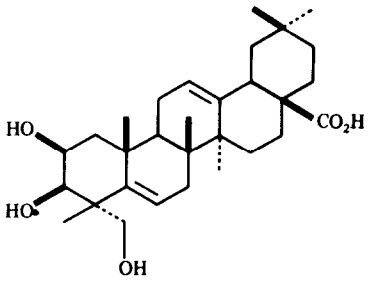


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Table 1. ^{13}C NMR chemical shifts of compound **1** and its derivatives in pyridine-d_5

C	1	4	2	3
1	46.3	46.5	47.4	43.2
2	70.7	70.9	71.8	70.8
3	83.0	82.8	73.0	73.0
4	43.8	43.9	43.6	45.5
5	49.0	49.1	49.1	148.7
6	67.5	67.4	67.0	120.9
7	41.1	41.1	41.0	33.2
8	39.3	39.2	39.3	37.5
9	48.7	48.7	49.0	46.0
10	36.8	36.7	37.1	38.5
11	24.1	24.1	24.1	24.1
12	123.5	123.0	123.1	123.4
13	143.5	144.3	144.2	145.1
14	42.8	42.8	42.9	43.1
15	28.2	28.3	28.2	27.7
16	23.2	23.8	23.8	23.6
17	47.4	46.7	46.7	47.0
18	41.8	42.1	42.1	42.5
19	46.4	46.5	46.5	45.8
20	30.9	31.0	31.0	31.0
21	34.2	34.3	34.2	34.2
22	32.7	33.3	33.3	33.1
23	65.4	65.2	67.5	69.4
24	16.7	16.8	16.2	21.3[a]
25	18.3[a]	18.5[a]	18.5[a]	23.0[a]
26	19.0[a]	18.9[a]	19.0[a]	23.8[a]
27	26.2	26.4	26.4	26.2
28	176.3	180.7	180.2	180.2
29	33.2	33.3	33.3	33.3
30	23.7	23.8	23.8	23.8
3-*O*-Glc				
1	105.5	105.7		
2	75.5	75.5		
3	78.5	78.5		
4	71.5	71.5		
5	78.1	78.2		
6	62.2	62.6		
28-*O*-Ara				
1	93.2			
2	76.1			
3	70.2			
4	66.1			
5	63.0			
Rha				
1	101.1			
2	72.4[b]			
3	72.6[b]			
4	83.5			
5	68.6			
6	18.6			
Xyl				
1	106.6			
2	75.3			
3	83.1			
4	69.2			
5	67.3			
Rha				
1	102.5			
2	72.6			
3	72.0[b]			
4	74.0			
5	69.8			
6	18.6			

[a,b]Signals may be interchangeable in each vertical column.

During this investigation, two minor antifungal saponins were purified by silica gel column chromatography. The ^{13}C NMR spectrum of these saponins not only showed the aglycone is protobassic acid, but also suggested that the structure of the oligosaccharide moiety is similar to Mi-saponin A. However, they differ from Mi-saponin A in that the monosaccharide apiose is present, suggesting their structures to be those of Mi-saponin B and its isomer.

EXPERIMENTAL

General. TLC was carried out on silica gel precoated Al sheets (Merck) with $CHCl_3$–MeOH–H_2O (13:7:1). Detection was with Godin reagent [14]. For normal phase CC, silica gel 60 (40–63 μm; Merck) was used.

Spectral data. NMR spectra were recorded at 50.1 MHz for ^{13}C and 200 MHz for ^1H in pyridine-d_5 (TMS as an int. standard). Negative FABMS was done on a VG Autospec instrument with thioglycerol as matrix.

Plant material. Clerodendrum wildii Moldenke was collected in November 1988 in Malawi.

Extraction and isolation. Powdered roots of *C. wildii* (193 g) were extracted with CH_2Cl_2 (3 × 1 l), followed by MeOH (3 × 1 l). The concentrated methanol extract (about 1 l) was cooled to −30° and filtered. The residual solid was dissolved in H_2O and extracted with *n*-BuOH. The filtrate (45.9 g) and *n*-BuOH extract (4.7 g) were combined, then chromatographed on Sephadex LH-20 (MeOH) to give three fractions. After CC of the first fraction on silica gel with $CHCl_3$–MeOH–H_2O (13:7:1), compound **1** (1.5 g) and a mixture of saponins (1.2 g) were obtained. Rechromatography of the mixture (300 mg) on silica gel using $CHCl_3$–MeOH–H_2O (6:4:1) afforded two pure compounds (140 and 80 mg).

Compound **1**. Powder; mp 232–235°; ^1H NMR δ: 0.91, 1.02, 1.26, 1.65, 1.99, 2.19 (each 3H, *s*), 1.69, 1.71 (each 3H, *d*, J = 7 Hz), 5.07 (1H, *d*, J = 7.3 Hz, Xyl H-1), 5.21 (1H, *d*, J = 6.8 Hz Glc H-1), 5.70 (1H, *s*, Ara H-1), 6.20, 6.45 (each 1H, *s*, Rha H-1). Negative-FABMS *m/z* (rel. int.): 1221 $[M-H]^-$ (100), 665 [aglycone + Glc $-H]^-$ (60).

Acid hydrolysis of compound **1**. A soln of **1** (211 mg) in 5% H_2SO_4–EtOH (1:1, 10 ml) was heated under reflux for 3 hr, then diluted with H_2O and extracted with EtOAc. The aqueous layer was neutralized with $BaCO_3$ to give glucose, arabinose, rhamnose and xylose, identified by TLC using EtOAc–HOAc–MeOH–H_2O (13:4:3:3), detection with *p*-anisidine phthalate. The EtOAc extract was sepd by CC on silica gel using $CHCl_3$–MeOH (9:1) to yield **2** (10 mg) and **3** (15 mg).

Protobassic acid (**2**). ^1H NMR δ: 0.94, 1.02, 1.30, 1.70, 2.07, 2.29 (each 3H, *s*), 3.37 (1H, *dd*, J = 12, 3 Hz), 4.06, 4.40 (each *d*, J = 10 Hz, H-23), 4.35 (1H, *d*, J = 4 Hz, H-3), 4.63 (1H, *br s*, H-2), 5.18 (1H, *br s*, H-6), 5.47 (1H, *br s*, OH), 5.62 (1H, *br t*, J = 3 Hz, H-12).

Bassic acid (**3**). ^1H NMR δ: 0.94, 1.02, 1.20, 1.22, 1.76, 1.77 (each 3H, *s*), 3.35 (1H, *br d*, J = 11 Hz, H-18), 4.08, 4.28 (each 1H, *d*, J = 10.5 Hz, H-23), 4.34 (1H, *d*, J = 3.7 Hz, H-3), 4.57 (1H, *q*, J = 3.5 Hz, H-2), 5.36 (1H, *br t*, J = 3 Hz, H-12), 5.91 (1H, *dd*, J = 3.2, 4.9 Hz, H-6).

Basic hydrolysis of compound **1**. A soln of **1** (203 mg) in 1 M KOH (8 ml) was heated under reflux for 2 hr. The reaction mixture was neutralized with 1 M HCl and extracted with *n*-BuOH. After CC on silica gel using $CHCl_3$–MeOH–H_2O (70:30:3), Mi-glycoside I (**4**) (41 mg) was obtained.

Mi-saponin I (**4**). ^1H NMR δ: 0.93, 1.01, 1.30, 1.62, 2.01, 2.21 (each *s*, 3H), 4.03, 4.59 (each 1H, *d*, J = 10.7 Hz, H-23), 4.39 (1H, *d*, J = 2.9 Hz, H-3), 4.90 (1H, *br s*, H-2), 5.15 (1H, *br s*, H-6), 5.58

(*br s*, H-12), 3.92 (1H, *m*, H-5'), 4.09 (1H, *t*, $J = 7.4$ Hz, H-2'), 4.17 (1H, *t*, $J = 7.4$ Hz, H-3'), 4.21 (1H, *t*, $J = 7.4$ Hz, H-4'), 4.47 (1H, *dd*, $J = 11.7$, 2.2 Hz, H-6'), 4.33 (1H, *dd*, $J = 11.7$, 5 Hz, H-6'), 5.23 (1H, *d*, $J = 7.4$ Hz, H-1').

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