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MOLLUSCICIDAL CONSTITUENTS OF THE STEM BARK OF *TETRAPLEURA TETRAPTERA*

O.D. Ngassapa[a], N.R. Farnsworth*[a], T.O. Henderson[b], C.W.W. Beecher[a] and G.L. Boye[c]
[a]Program for Collaborative Research in the Pharmaceutical Sciences, College of Pharmacy, University of Illinois at Chicago, Box 6998, Chicago, IL. 60680 USA. [b]Department of Biological Chemistry, University of Illinois College of Medicine at Chicago, Chicago, IL. USA and [c]Department of Pharmacology, Medical School, University of Ghana, Accra, Ghana

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ABSTRACT. Two triterpene glycosides have been isolated from the stem bark of *Tetrapleura tetraptera* by a bioassy-directed fractionation procedure. Compound **2**; 3-O-[β-D-glucopyranosyl-2'-acetamido-2'-deoxy]-echinocystic acid, is a new natural product, while compound **1**, aridanin, is a known compound. Compounds **1** and **2** are 100% lethal for *Biomphalaria glabrata* snails at 1.25 and 5.0 ppm, respectively.

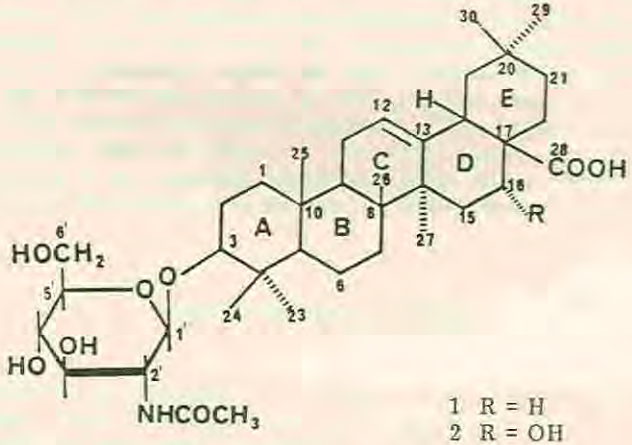
INTRODUCTION

Tetrapleura tetraptera Taub. (Leguminosae) has been reported to be a potent molluscicide (1-3); the activity of which has been attributed to saponins and coumarins (1). Previous phytochemical investigations (4) of the fruit led to the isolation of aridanin, **1**, and other known compounds. Aridanin has been previously isolated from the leaves of *Pithecellobium cubense* Bisse and *P. arboreum* L. (Leguminosae) (5). Recently, Adewunmi *et. al.* (6,7) reported on the effects of aridanin on *Biomphalaria glabrata* Say and *Lymnaea columella* Say. In our preliminary investigation the stem bark of *T. tetraptera* showed a stronger molluscicidal activity than the fruit; therefore, we carried out the investigation of the stem bark in order to isolate and identify the molluscicidal constituents of this plant part. We report here the isolation and identification of another active triterpene glycoside, **2**, which has been obtained along with aridanin. Glycoside **2** had been previously obtained as a product of partial hydrolysis of Entada-saponin-III (8), a saponin of *Entada phaseoloides* (L.) Merrill, (Leguminosae).

RESULTS AND DISCUSSION

The methanol extract of the stem bark of *T. tetraptera* was partitioned between water and 1-butanol. The butanol phase, after repeated column chromatography, yielded aridanin, **1**; 3-O-[β-D-glucopyranosyl-2'-acetamido-2'-deoxy]-oleanolic acid and **2**; 3-O-[β-D-glucopyranosyl-2'-acetamido-2'-deoxy] -echinocystic acid. The two compounds are closely related, the only difference is the presence of a 16α-hydroxyl group in **2**. Both compounds are insoluble in water, sparingly soluble in chloroform and methanol, and soluble in pyridine. Compound **2** is more polar than **1**; the Rf values are, respectively, 0.43 & 0.48 (solvent system I) and 0.49 & 0.53 (solvent system II). They were identified by FABMS, EIMS, IR,

^{1}H NMR and ^{13}C NMR (broad-band decoupled, APT & SFORD) spectra. FABMS confirmed the molecular weight of **1** to be 659 and that of **2** to be 675, since quasi-molecular ions, $[M + H]^+$, were observed at m/z 660 and m/z 676 for **1** and **2**, respectively. EIMS of **2** showed fragment ions at m/z 264 and 246 which are a result of the retro-Diels-Alder fragmentation in ring C of the aglycone part and subsequent loss of a water molecule. This is in agreement with the presence of a C-12,13 double bond and a hydroxyl group in either ring D or E. The presence of secondary 16α-OH is further supported by ^{1}H NMR, with H-16 appearing at 5.25 ppm, and ^{13}C NMR, with C-16 at 75.06 ppm. This is in agreement with the previously reported data (8) for this compound, and the ^{13}C NMR data reported for other 16α-hydroxy oleanene-type triterpenoids (9,10). The IR spectra of **1** and **2** are almost identical, except for a slight difference in the fingerprint regions. This indicates that the two compounds have similar functional groups, namely:- -OH, -COOH, & -NHCO-. Both **1** & **2** are monodesmosidic glycosides, with the sugar moiety attached to the aglycone at C-3 only (89.10 & 89.51 ppm, respectively). Both ^{1}H NMR and ^{13}C NMR indicate that the type of sugar is the same in the two glycosides (see Table 2). Fig. 1 shows a 2D COSY spectrum of **1**; correlations between -NH-& H-2', and H-1' & H-2' are clearly observed. From this sepctrum, it should be noted that in one of the previous reports (4) on **1**, the positions (^{1}H NMR) of H-2' & H-3' had been interchanged. Therefore, the positions of sugar protons should be as shown in Fig. 1. The 2D COSY of **2** also shows a similar coupling pattern in the sugar region as that of **1**. The β-configuration of the sugar is confirmed by the large coupling constant ($J_{1',2'}$ = 8.3 Hz) of the anomeric protons, (H-1') which appear as doublets at 5.07 and 5.06 ppm. It is further supported by the chemical shifts of anomeric carbons, at 104.90 and 105.16 ppm. From these data and by comparison with those in literature (4,5,8) it is confirmed that the structures of **1** and **2** correspond to 3-O-[β-D-glucopyranosyl-2'-deoxy]-oleanolic acid and 3-O-[β-D-glucopyranosyl-2'-acetamido-2'-deoxy]-echinocystic acid.



1 R = H
2 R = OH

We confirm the molluscicidal activity of **1**, aridanin; our findings (see Table 1) indicate that it is 100% lethal for *Biomphalaria glabrata* Say at 1.25 ppm. The slight difference in the level of activity from that which has been previously reported (6,7) could be due to some differences in the experimental conditions. The molluscicidal activity of **2** is reported now for the first time; it exhibits 100% mortality at 15.0 ppm. Signs of haemolysis were observed in test solutions containing **1** or **2**. It is probably due to the disruption of snail

membranes. From the results (Table 1) it seems that the presence of a 16α-hydroxyl group in ring D reduces the molluscicidal activity by about ten times. Aridanin is among the most active natural products which have been, so far, tested for molluscicidal activity. However, it is about ten times less active than the reference molluscicide, Bayluscide (70% niclosamide), whereas 2 is about a hundred times less active.

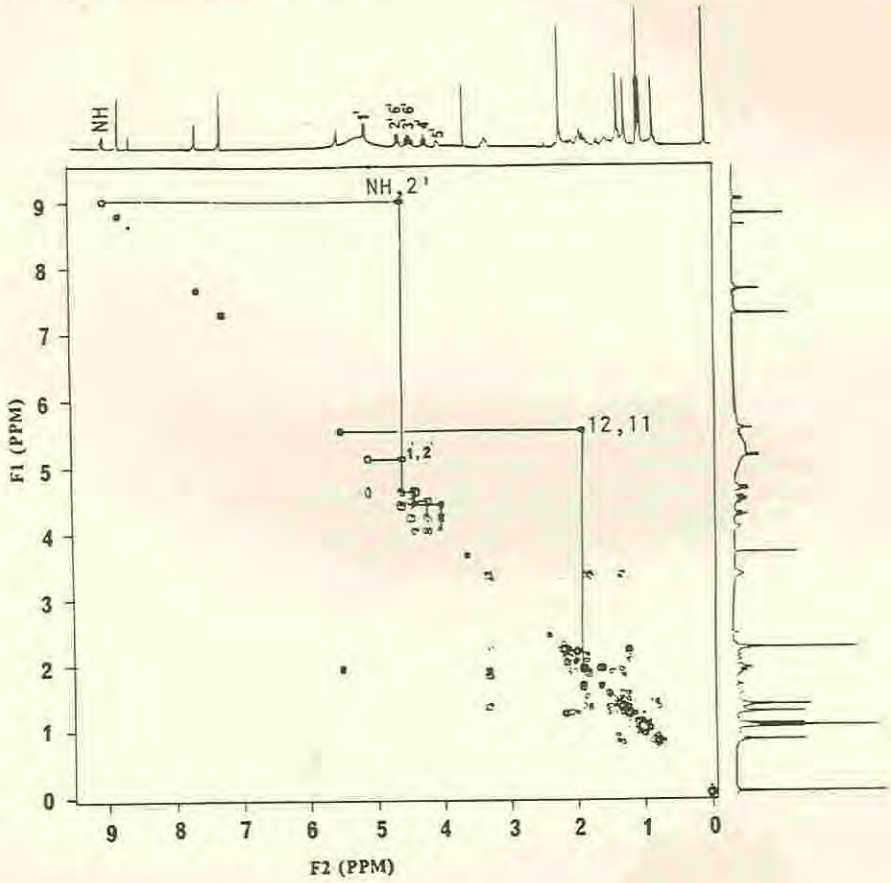


Fig. 1 2D ^{1}H-^{1}H COSY Spectrum of 1

EXPERIMENTAL

General. Melting points were determined on a Kofler-type hot-stage apparatus and are uncorrected. IR spectra were taken as KBr pellets on a Nicolet MX-1 interferometer, while optical rotations were measured with a Perkin-Elmer Model 241 polarimeter. EIMS was run on a Varian MAT 112S double focussing mass spectrometer at 80 eV. FABMS was run on a Finnigan MAT 90 mass spectrometer. ^{1}H NMR spectra (in pyridine-d_5, TMS int. standard) were determined on Varian XL-300 (300 MHz) and Nicolet NMC 360 (360 MHz) spectrometers. ^{13}C NMR spectra were measured at 90.8 MHz on a Nicolet NMC 360 spectrometer. Column chromatography was run on Si gel for flash

Table 1. Molluscicidal activity of 1 & 2 on *B. glabrata* at 24 hr exposure.

1		2	
Concn (ppm)	% mortality	Concn (ppm)	% mortality
0.20	0	5.0	0
0.50	20	7.5	40
0.75	30	10.0	90
1.00	80	15.0	100
1.25	100		

1 ppm = 1 mg/1; no snail died in 1% MeOH solution;
0.2 ppm Bayluscide showed 100% mortality

column chromatography (40 μm, J.T. Baker Chemical Co., Phillipsburg, New Jersey, USA). TLC was performed on Si gel 60 F_{254} using solvent system I, $CHCl_3$-MeOH (7:3) and II, $CHCl_3$-MeOH-water (13:7:2, lower phase). TLC spots were detected by spraying with 1% vanillin in conc. sulphuric acid, and subsequent heating of TLC plates at 120°C.

Bioassy. Biomphalaria glabrata snail used in this study (albino Puerto Rican strain) were originally obtained through the National Institute of Allergy and Infectious Diseases, NIH, and maintained in one of our laboratories since 1981. The average shell diameter of the test snails was 12 mm. Tests were done in duplicate using five snails for each test. Test solutions were prepared from stock solutions in methanol by dilution with dechlorinated tap water to give 100 ml containing varying amounts of the test compound. Water temperature was maintained at 21-22°C; Bayluscide and 1% methanol in aged, dechlorinated tap water were used for positive and negative controls, respectively. The snails were exposed to the test compound by immersion in the solutions for a period of 24 hr, then they were rinsed three times with dechlorinated tap water and left to recover in the same for a further 24 hr, when the deaths were recorded. Death was ascertained by examining immobilized snails under a dissecting microscope for the absence of heart beat.

Extration and isolation. The plant material was supplied by the Centre for Scientific Research into Plant Medicines, Mampong Akwapim, Ghana, in June 1987. It was identified by the staff of that Centre and voucher specimens have been deposited with the herbarium of the Field Museum of Natural History, Chicago, U.S.A.

All solvents used in the extraction and isolation process were re-distilled. Dried and ground stem bark (3.5 kg) was subjected to exhaustive and successive extraction at room temperature, with pet-ether, chloroform, methanol and water. Each of the extracts was tested for molluscicidal activity at 10, 20 & 100 ppm. The methanol extract showed the highest activity, killing 90% of the snails at 100 ppm after a 24 hr exposure period. Hence, subsequent investigation was carried out using this extract. The extract (310 g) was suspended in distilled water (2 l), and extracted with 1-butanol (3x1 l). Butanol was removed *in vacuo*, at 50°C, and the residue was column chromatographed and eluted with $CHCl_3$-pet-ether, then $CHCl_3$-MeOH mixtures of increasing polarity. Fractions, 500 ml each, were collected and pooled based on TLC results. Compound **1** separated from fraction 34 following concentration to a small volume, while combined fractions 35-38 gave a mixture of **1**, **2** and other more polar compounds. Rechromatography of this mixture with $CHCl_3$-MeOH mixtures gave a fraction containing **1** and **2**. Repeated column chromatography using $CHCl_3$-MeOH-H_2O (80:20:5, lower phase) led to the separation of **1** and **2**.

Table 2. ^{13}C-NMR chemical shifts (δ, ppm) of 1 & 2 in pyridine-d_5.

Carbon	1	2	Mult.	Carbon	1	2	Mult.
1	38.54	38.98	t	20	30.97	31.36	s
2	26.37	26.71	t	21	34.23	36.47	t
3	89.10	89.51	d	22	33.19	33.12	t
4	39.25	39.56	s	23	28.15	28.47	q
5	55.72	56.18	d	24	17.00	17.33	q
6	18.52	18.88	t	25	15.38	15.82	q
7	33.19	33.84	t	26	17.37	17.79	q
8	39.72	40.22	s	27	26.20	27.57	q
9	47.98	47.50	d	28	180.09	180.39	s
10	36.95	37.33	s	29	33.29	33.68	q
11	23.67	24.12	t	30	23.76	25.10	q
12	122.50	122.67	d	1'	104.90	105.16	d
13	144.76	145.44	s	2'	58.05	58.35	d
14	42.14	42.44	s	3'	76.15	76.45	d
15	28.31	36.52	t	4'	72.64	72.98	d
16	23.67	75.06	t,d	5'	78.29	78.52	d
17	46.65	49.23	s	6'	62.98	63.31	t
18	41.92	41.78	d	-NHCO-	170.09	170.57	s
19	46.47	47.62	t	$-COCH_3$	23.76	24.01	q

Aridanin, 1. Yield 877 mg (ca. 0.025%), colourless needles (MeOH), m.p. 274-5°, lit. (4) 276-280 °C, $[\alpha]^{25}_D$ + 35° (pyridine, c 0.1), lit. (4) + 40.96°. FABMS, +ve ion mode, glycerol matrix, m/z 660; $[M + H]^+$, EIMS, m/z (rel. int.) 456(1); [659 - N-acetyl glucosy]$^+$, 439(3), 248(100); $[C_{16}H_{24}O_2]^+$, 203(78); [248-COOH]$^+$, 190(20), 133(19), 69(43), 43(77). IR, ν_{max} (cm^{-1}), 3600-2400 [O-H], 2946-2880 [C-H, aliph.], 1685 [-COOH], 1664 & 1547 [-CONH-], 1125-1000 [C-O]. ^1H NMR (δ, ppm), 0.78, 0.96, 0.99, 1.02, 1.20, 1.31 (21H, s, 7 x CH_3), 2.17 (3H, s, $-COCH_3$), 5.07 (1H, d, H-1', $J_{1',2'}$ = 8.3 Hz), 5.42 (1H, br, H-12), 8.92 (1H, d, -NH-, $J_{NH,2'}$ = 9.0 Hz).

Compound 2. Yield 287 mg (ca. 0.008%), colourless plates (MeOH-acetone), m.p.* 252-5°, lit. (8) 277-280 °C, $[\alpha]^{25}_D$ + 18° (EtOH, c 0.1), lit. (8) + 7.3°. FABMS, +ve ion mode, glycerol matrix, m/z 676; $[M + H]^+$, EIMS, m/z (rel. int.), 613(1); 675-H_2O-$CO_2]^+$, 410(10), 246(6); $[C_{16}H_{24}O_3]^+$, 246(13); [264-H_2O]$^+$, 202(24), 189(24), 187(34), 131(22), 69(59), 43(100). IR ν_{max} (cm^{-1}), 3600-2500 [O-H], 2950-2870 [C-H, alph.], 1690 [-COOH], 1663 & 1550 [-CONH-], 1120-1000 [C-O]. ^1H NMR (δ, ppm), 0.80, 0.99, 1.02, 1.06, 1.19, 1.85 (21H, s, 7 x CH_3), 2.17 (3H, s, $-COCH_3$), 5.06 (1H, d, H-1', $J_{1',2'}$ = 8.3 Hz), 5.25 (1H, br, H-16), 5.65 (1H, br, H-12), 8.95 (1H, d, -NH-, $J_{NH,2'}$ = 8.7 Hz).

*The difference between the observed and reported m.p. range & $[\alpha]^{25}_D$ values of 2, cannot be explained at the moment.

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