Jpn. J. Infect. Dis., 53, 17-19, 2000

Short Communication

Molluscicidal Saponins from *Anagallis arvensis* against Schistosome Intermediate Hosts

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(Received January 26, 2000. Accepted March 10, 2000)

SUMMARY: The molluscicidal activity of saponins isolated from the plant *Anagallis arvensis* (Primulaceae) was studied against schistosome intermediate hosts, *Biomphalaria glabrata* and *Oncomelania quadrasi*. Strong molluscicidal activity was found in two compounds called desglucoanagalloside B and anagalloside B. Their structures were identified on the basis of chemical and spectroscopic analyses and their activities are comparable to that of the synthetic molluscicide, niclosamide.

Schistosomiasis, the most significant trematode disease of human, is a world-wide health problem. It affects 200 million people and poses a threat to 400 million more in 74 countries around the world (1). In Egypt, newly constructed dams and irrigation systems may have changed the ecological conditions and contributed to the sudden and unexpected increase in the intermediate host snails of schistosomes. Human infection with *Schistosoma mansoni* is spreading and increasing in association with the use of perennial irrigation (2). There remains a need for determining the molluscicidal allelochemicals from plants in the hope that they might provide cheap, locally producible, biodegradable, and effective control agents in the endemic areas (3,4). Several local desert and cultivated plants have been screened in the laboratory and under field conditions against the snail vectors in Egypt. Among them, *Anagallis arvensis* is considered one of the most promising molluscicidal plants, and its saponin fraction has been reported to express high molluscicidal activity (5). In this paper we describe the isolation and structure determination of the molluscicidal saponins of *A. arvensis* and their molluscicidal activities.

The bioassay was performed on two species, *Biomphalaria glabrata* (Puerto Rico origin, the intermediate host of *S. mansoni*) and *Oncomelania quadrasi* (Mindanao, Philippines origin, the intermediate host of *Schistosoma japonicum*), both maintained in the laboratory of the Department of Parasitology, National Institute of Infectious Diseases in Tokyo. Snails used in the experiments were young-mature (8.5 to 10.5 mm shell diameter for *B. glabrata*; 4.0 to 4.5 mm shell height for *O. quadrasi*). The tests were carried out as follows: 10 snails in separate plastic dishes (diameter, 60 mm and height, 30 mm) were exposed to a serial dilution of a known concentration of test samples in 60 ml of distilled water at a temperature of 28°C for 24 h. After exposure, the snails were washed in distilled water and their shells were crushed for examination. Visual observation using a microscope was performed to determine whether the snail was alive or dead according to the WHO criterion (6). The powder-like sample obtained by lyophilization from each stage of the isolation process was tested for activity at various concentrations. Niclosamide (ICN Biomedicals Inc., Aurora, Ohio, USA) was used as a reference molluscicide. This synthetic reagent has been in use as a molluscicide since the 1960s and remains the molluscicide of choice (1).

The plant, *A. arvensis*, was collected and deposited at the Laboratory of Medical Chemistry, Theodor Bilharz Research Institute, Giza, Egypt. The material was dried in the shade and finely powdered for use. The fractionation scheme for the isolation of the molluscicidal saponins is shown in Figure 1. Briefly, dried whole plants of *A. arvensis* (25 g) were extracted using methanol. The methanol extract was partitioned between ethyl acetate and water. The water-soluble fraction was then chromatographed on silica gel stepwise and eluted with an increasing ratio of methanol in ethyl acetate. The molluscicidal activity was found in the 25-35% methanol eluate. Further column chromatography on silica gel using an upper phase of ethyl acetate-water-methanol (10:3:2) gave two active fractions, Fr. A-3 and Fr. A-5 (Fig. 1). They were separately submitted to separation on an ODS gel eluted with methanol-water (3:1). Final purification of the active fractions by preparative HPLC (YMC GEL ODS-A 60-400/230, 8.0 ml/min) gave active compounds 1 (34.7 mg) and 2 (15.1 mg).

Compound 1, $[\alpha]^{20}_{D}$ −1.5° (c=0.87, methanol), showed an apparent ion peak at *m/z* 1063.5689$[MH]^+$ in the HR FAB-MS, showing the molecular formula of $C_{52}H_{87}O_{22}$ (calculated for MH^+:1063.5540). Compound 2, $[\alpha]^{20}_{D}$ −4.2° (c=0.38, methanol), also showed an apparent ion peak at *m/z* 1225.6217 $[MH]^+$ in the HR FAB-MS, showing the molecular formula of $C_{58}H_{97}O_{27}$ (calculated for MH^+:1225.6223). Inspection of the ^{1}H- and ^{13}C-nuclear magnetic resonance (^{1}H- and ^{13}C-

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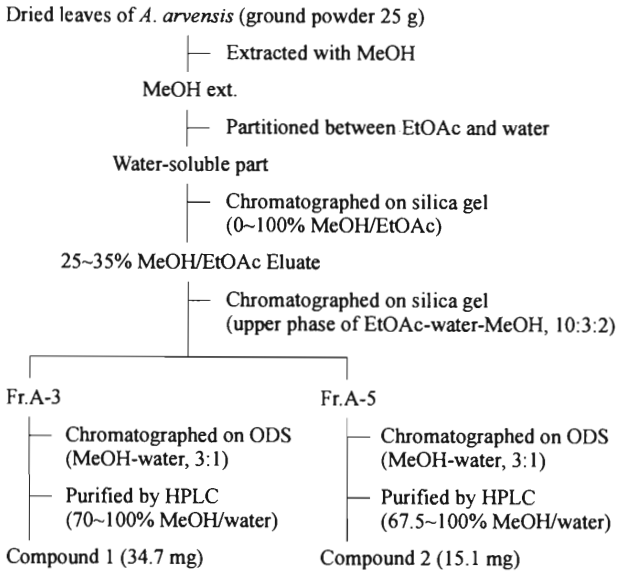

Fig. 1. The fractionation scheme for isolation of the molluscicidal saponins.

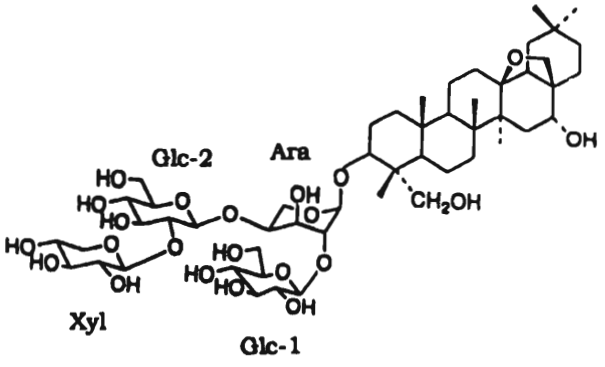

Fig. 2. Structure of Compound 1 (Desglucoanagalloside B)

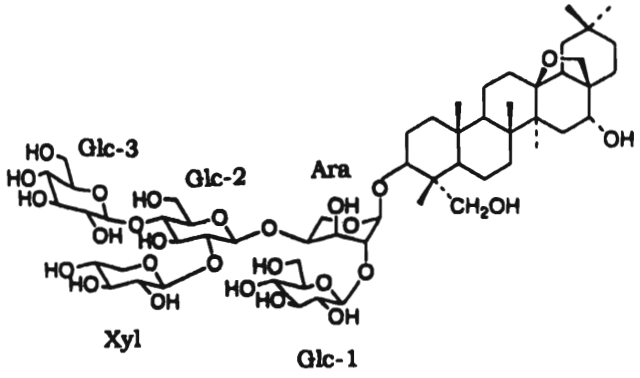

Fig. 3. Structure of Compound 2 (Anagalloside B)

NMR) spectra (Table 1) indicated that compounds 1 and 2 were in close agreement with those of desglucoanagalloside B (Fig. 2) and anagalloside B (Fig. 3) reported by Shoji et al. (7).

In preliminary examinations, the two compounds at concentrations of 5 mg/l (ppm) were found to be lethal to both types of snails. Further observations are summarized in Table 2. Compound 1, called desglucoanagalloside B, was found to be active against *B. glabrata* snails at concentra-

Table 1. ^{1}H and ^{13}C NMR data of compound 1 and 2

	Compound 1		Compound 2	
Position	δ ^{1}H!	δ ^{13}C	δ ^{1}H!	δ ^{13}C
1		39.3		39.2
2		26.0		26.0
3		82.5		82.5
4		43.7		43.7
5		48.0		48.0
6		17.8		17.7
7		34.2		34.2
8		42.5		42.5
9		50.7		50.7
10		36.9		36.9
11		19.4		19.4
12		33.0		33.0
13		86.5		86.5
14		44.7		44.6
15	2.23!! (14.5 & 5.0)	37.1	2.25!! (14.5 & 5.0)	37.1
16		77.2		77.2
17		44.7		44.6
18		51.6		51.6
19	2.74!! (13 & 13)	39.0	2.75!! (12 & 12)	39.0
20		31.7		31.9
21	2.54!! (13 & 4.9)	36.9	2.58!! (13 & 4.9)	36.9
22		31.9		31.9
23	3.70, 4.22 (10.5)	65.0	ca. 3.7, 4.3 (ca. 10)	65.0
24	1.06	13.3	1.06	13.3
25	0.96	17.1	0.95	17.1
26	1.38	18.6	1.36	18.6
27	1.47	19.6	1.47	19.6
28	3.31, 3.60 (7.0)	78.0	3.32, 3.61 (7.4)	78.1*
29	1.08	33.8	1.07	33.8
30	0.98	24.8	0.98	24.8
sugar moiety				
3-*O*-Ara				
1	4.9 - 5.0	104.1	4.91 (7.8)	103.8
2		80.3		80.2
3		73.4		73.2
4		78.2		78.4**
5		64.2		63.9
3-*O*-Glc-1				
1	5.48 (7.6)	105.2	5.47 (7.6)	104.8
2		76.2		76.2
3		78.3		78.4**
4		71.5		71.5
5		78.1		78.2*
6		62.7		62.7
3-*O*-Glc-2				
1	5.03 (5.0)	103.7	5.04 (5.7)	103.7
2		85.4		83.8
3		77.7		74.8
4		71.1		80.3
5		78.3		76.4
6		62.4		61.6
3-*O*-Xyl				
1	5.06 (7.7)	107.7	5.00 (7.5)	107.2
2		76.3		76.0
3		77.8		77.4
4		70.7		70.7
5		67.5		67.3
3-*O*-Glc-3				
1			5.16 (7.9)	105.1
2				75.8
3				78.1*
4				71.6
5				78.3**
6				62.4

The spectra were measured in d5-pyridine at 500 MHz for ^{1}H and 125 MTz for ^{13}C using TMS as an internal standard, and expressed in ppm.

I : Only the signals clearly assinable are shown, and the values in parethesis are coupling constants in Hz.

II: The signal corresponds to one of the methylene protons.

* and **: The signals may be interchangeable.

Table 2. Molluscicidal activity of purified two saponins from *Anagalis arvensis* on *Biomphalaria glabrata* and *Oncomelania quadrasi*

	Biomphalaria glabrata						
	10 ppm	5 ppm	2.5 ppm	1.25 ppm	0.625 ppm	0.3125 ppm	0 ppm
Desglucoanagalloside B	10/10	10/10	10/10	6/10	1/10	0/10	0/10
Anagalloside B	10/10	10/10	8/10	0/10	0/10	0/10	0/10
Niclosamide	10/10	10/10	5/10	1/10	1/10	1/10	0/10
	Oncomelania quadorasi						
	10 ppm	5 ppm	2.5 ppm	1.25 ppm	0.625 ppm	0.3125 ppm	0 ppm
Desglucoanagalloside B	10/10	10/10	10/10	10/10	8/10	0/10	0/10
Anagalloside B	10/10	10/10	10/10	6/10	4/10	0/10	0/10
Niclosamide	10/10	10/10	10/10	10/10	10/10	7/10	0/10

Number of dead snails/Number of tested snails

tions of 0.625 ppm and over, and lethal to all tested snails at concentration of 2.5 ppm. At concentrations of 1.25 ppm and over, 100% of the tested *O. quadrasi* snails were killed. The molluscicidal activity of desglucoanagalloside B is comparable to that of the synthetic molluscicide, niclosamide. Compound 2 (anagalloside B) might be less effective than compound 1. In the case of anagalloside B, the tested *B. glabrata* and *O. quadrasi* snails showed 100% mortality rates at concentrations of 5 ppm and 2.5 ppm, respectively.

It is apparent that *A. arvensis* can be a candidate molluscicidal plant not only in Egypt but also in other areas endemic with schistosomiasis because this plant is widely distributed from the tropics to the temperate regions around the world.

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