Supporting information

***Rec. Nat. Prod*. 6:3 (2012) 301-305**

Potent Insecticidal Secondary Metabolites from the Medicinal Plant *Acanthus montanus*

Elham Amin,[1, 5] Mohamed M. Radwan,[3,5] Seham S. El-Hawary,[2] Magda M. Fathy,[2] Rabab Mohammed,[1] James J. Becnel,[4] and Ikhlas Khan[e]

[1]Faculty of Pharmacy, Beni Suef University, Beni Suef, Egypt

[2]Faculty of Pharmacy, Cairo University, Cairo, Egypt

[3]Faculty of Pharmacy, Alexandria University, Alexxandria, Egypt.

[4]USDA, ARS, Center for Medical, Agricultural, and Veterinary Entomology Gainesville, FL 32608 USA.

[5]National Center for Natural Products Research, University of Mississippi, University, MS 38677, USA.

1. General procedures

1.1. Phytochemical study

HR-ESI-MS data were obtained on an Agilent Series 1100 SL mass spectrometer. NMR spectra were recorded on Varian AS 400 NMR spectrometers. IR spectra were recorded on a Bruker Tensor 27 spectrophotometer. Specific rotations were measured at ambient temperature using a Rudolph Research Analytical Autopol IV automatic polarimeter. Flash column chromatography was done by using Biotage SP-1. HPLC was done using a Delta Prep 4000 (Waters Corporation) equipped with a dual wavelength detector Model 2487 adjusted at 210 and 254 nm. The preparative HPLC columns were from Phenomenx Luna C_8 and C_{18} (100 A 250 x 15.00, 5µ). The HPLC methods were: Method 1; C_{18} column, using H_2O+0.05% formic acid(W) and MeOH+0.05% formic acid(M) in a gradient mode: W/M 65/35 for 10 min, 65/35 -50/50 for 10 min, 50/50-0/100 for 20 min, and method 2; C_8 column, using H_2O+0.05% formic acid(W) and ACN+0.05% formic acid(N) in a gradient mode: W/N 65/35 for 10 min, 65/35 -50/50 for 5 min, 50/50-0/100 for 15 min. Gravity column chromatography was performed by using (J.T. Baker 40 μm for flash chromatography). Thin layer chromatography (TLC) was carried out on

silica gel 60 F_{254} and RP-C_{18} F_{254} plates (Merk). Sep-Pak cartridges (C_{18}, 60 mL, 10g) were purchased from Supelco. Standard sugar samples were purchased from Sigma-Aldrich.

1.2. Biological study

Mosquitoes: The Gainesville strain of *Aedes aegypti* (established 1952) were reared in the insectary of the Mosquito and Fly Research Unit at Center for Medical, Agricultural, and Veterinary Entomology (CMAVE), USDA-ARS, Gainesville, Florida. Standard rearing protocols previously described were followed [25-26].

Adult bioassays: Assays followed the previously established protocols [26]. Briefly, three to seven days old adult females *A. aegypti,* were anesthetized by CO_2 and sorted into clear 100 cc polystyrene cups, covered with two layers of nylon tulle. The mosquitoes were provided with a cotton ball soaked with 10% sucrose and allowed to recover for at least 16h prior to testing. A total of 10 females were used for each concentration. Permethrin (55% cis and 45% trans, Chem Service, Inc. West Chester, PA, USA) was used as a positive control. Eight of the isolated nine compounds were tested for adulticidal activity. Test samples were adjusted to 10% (in methanol), then serially-diluted in acetone to obtain the testing concentrations. For application, the mosquitoes were anesthetized using CO_2 for 15 seconds, and then treated with 0.5 ul of test solution applied to the thorax of each insect using a repeating syringe (Hamilton Co., Reno, NV). Adults were held in a 27°C 16:8 (L:D) chamber for 24h and then scored for mortality.

2. Extraction and isolation

Aerial parts of the plant (400 g) were finely ground and extracted with 80% MeOH (8x500ml). The extract was evaporated under reduced pressure at 45°C, to give 30 g dry residue. The extract (30 g) was chromatographed over VLC (Si, 1Kg, 30x3.5 cm) using *n*-Hexane (1L), 20% Ethyl acetate/*n*-Hexane (1L), 40% (1L), 60% (1L), 80% (1L), 100% Ethylacetate, 10% MeOH/Ethylacetate (1L), 20% (1L), 40% (1L), 60% (1L), 80% (1L), and 100% MeOH (3L). The similar fractions were pooled together to give four main fractions, A, B, C, and D.

Fraction A (400mg) was applied to SPE cartridge (C_{18}, 60ml, 10g), 90% aqueous MeOH (500 ml) to give 2 (18.0 mg). Frs. 17-24 was identified using GC to be a mixture of linoleic, oleic and stearic acids.

Fraction B (500 mg) was chromatographed using Biotage Sp-1 (25+M, Si) column and $CHCl_3$ /MeOH (90/10, 500 ml). Frs. 9-44 (240mg) was dissolved in least amount of MeOH (3ml), and filtered, the insoluble part was washed several times with MeOH and dried to give 1 (20.0mg).

Fraction C (400 mg) was subjected to flash chromatography over (40+M, C_{18}) column, using MeOH/H_2O isocratic development (3/7, 2L), to give two main fractions C_1 and C_2. C_1 was then purified on RP-HPLC using method 1, to give 3 (R_t = 31.4 min, 10.0 mg). C_2 upon RP-HPLC purification using method 2, gave 4 (R_t =14.8 min, 4.5 mg) and 5 (R_t =15.9 min, 5.4 mg).

Fraction D (4.5 g), was subjected to flash chromatography, using (40+M, Si) column and $CHCl_3$/MeOH gradient, 90/10 (300 ml), 8/2 (500ml), 7/3 (500ml), 1/1 (500ml), and MeOH 100% (1L), to give two main fractions, D_1 and D_2. Subfraction D_2 (562 mg) was chromatographed using, (40+M, C_{18}) column and MeOH/H_2O gradient: 1/9 (300ml), 2/8 (200 ml), 3/7 (500 ml), 5/5 (500 ml), and 10/0 (1L) to give 6 (50.0mg) and 7 (40.0mg). Fraction D_1 was flash chromatographed using (40+M, C_{18}) column and MeOH/H_2O isocratic development (30/70, 2L), to give two main subfractions; I and II. Subfration I was subjected to HPLC (method 1) to give 8 (2.5mg). Subfration II was purified using HPLC (method 1) to give 9 (10.0mg)