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Short Communication

Insecticidal activity of 3-acetyl moraldehyde and agauriasterone from *Agauria salicifolia*

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Bioassay guided chromatographic separation of a methanolic extract of *Agauria salicifolia* led to isolation of 3-acetyl moraldehyde and aguariasterone, whose structures were elucidated using spectroscopic data (UV, IR, and NMR) and melting points. Insecticidal activity was done using adult *Phaedon cochleariae* (Mustard beetle) at concentration of 1, 0.1, and 0.01% at a dose of 1.00 µg per insect of the compound. 3-Acetyl moraldehyde and aguariasterone were found to be active with LD_{50} values of 0.04 and 0.15 µg, respectively.

Key words: *Agauria salicifolia*, *Phaedon cochleariae*, 3-Acetyl moraldehyde, agauriasterone.

INTRODUCTION

Despite enormous expenditure on agrochemicals, pests reportedly destroy between 13 – 16% of the global food production with yield of about $90 billions each year; hence the protection of food supply from pests is a major worldwide problem (Leonard and Geoffrey, 1998). To prevent economically unacceptable losses of yield and quality of agricultural crops caused by insects, protection of crops with agrochemicals is necessary.

Currently synthetic pesticides are losing their effectiveness and to date, hundreds of pest species have developed resistance to at least one pesticide formulation and a dozen or so species are immune to all. Furthermore synthetic pesticides are often very persistent in soil and water (especially halogenated hydrocarbons) and can accumulate in the food chain hence threatening the health of the entire ecosystem (Coaker, 1994; Thomson, 1998).

According to World Health Organization estimates, up to 20,000 people die of pesticides poisoning in the third world countries each year. This has sparked growing public concerns of undesirable effects of pesticides on human health and environment. This has led to the withdrawals of some commercial pesticides, increasing the vulnerability of crops to pests and diseases (Brucechwatt, 1971). There is therefore need to develop alternative environmentally friendly, non toxic and effective pesticides for the control of food pesticides. One way of developing safe insecticides is by screening the traditional botanical pesticides. As part of ongoing research on new insecticides, *Agauria salicifolia* (Ericaceae) was screened for insecticidal activity. The plant is known to be toxic to herbivores especially goats. The roots and the bark of the stem are used to cure several ailments including snake bites, skin diseases, added to bait to kill rats (Barry, 1937).

This study led to isolation of 3-acetylmoraldehyde (1) and aguariasterone (2), which were investigated for insecticidal activity against adult *Phaedon cochleariae*.

EXPERIMENTAL

The melting points were determined using an electrothermal melting point apparatus with a thermometer range of 0 – 360°C and was uncorrected. Infrared spectrums were run on a Nicolet Impact 410 FT-IR spectrometer with the compound in chloroform.

^{1}H and ^{13}C NMR analysis were done using a JOEL DRX500 (500-125.75 MHz) NMR spectrometer. The solvent used was deuteriated chloroform with TMS as the internal standard. Ultraviolet spectrums were run using a Shimadzu UV-160A UV-VIS speectrometer using chloroform as solvent. Electron impact mass measure-

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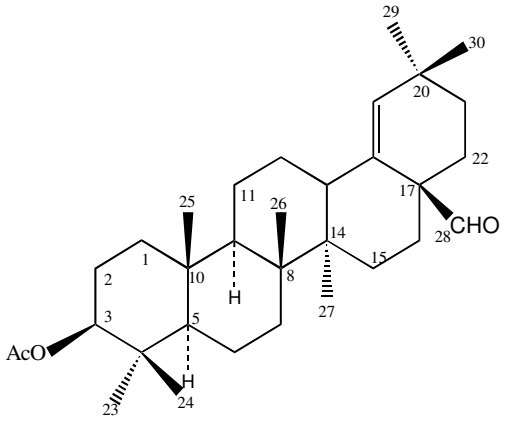

Figure 1. 3-acetyl moraldehyde (1).

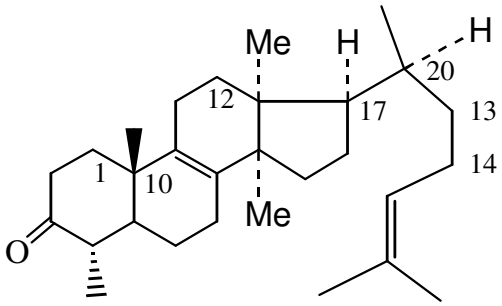

Figure 2. Aguariasterone (2).

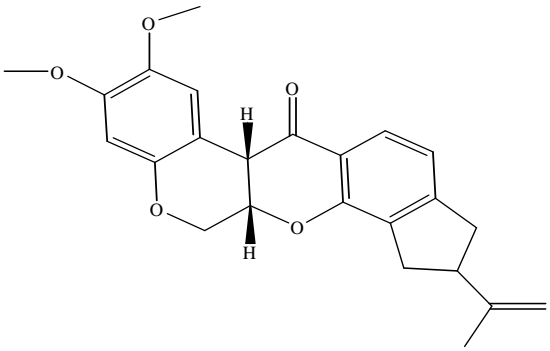

Figure 3. Rotenone (3).

ments were recorded on a VG Autospec mass spectrometer at 70 eV. HPLC was done using Gilson equipment and peaks monitored using Holochrome UV detector set at 290 nm, for analytical HPLC, synergi 4µ Hydro – RP 80A column (size 150 by 4.6 mm) was used and Hichron KR 100 5C18 – 25074 columns were used for semi-preparative HPLC. Potentiometric chart recorder operating at 10 mV at a speed of 5 mm/s was used as a recorder. Dry column chromatography was carried out using 5-40 µm silica gels and flash chromatography was done using 220-440 mesh flash silica gels.

P. coehleariae (adult mustard beetle) were used for insecticidal test and were obtained from insect colony of Rothamsted research Limited. The stem bark of *A. salicifolia* was collected from Uasin-Gishu district, 320 Km West of Nairobi, Kenya. The staff of Botany Department Herbarium, Moi University, where a voucher specimen was deposited, identified the plant.

Solvent extraction and chromatography

The stem bark of the plant were chopped into small pieces, air dried at room temperature for three weeks, then ground into a powder. One Kilogram of the powder was soaked in cold methanol for 3 days to extract the compounds. The resulting brown filtrate was concentrated under reduced pressure using Buchi R110 rotatory evaporator to a dark brown semi-solid, 5 g of which was subjected to solvent partitioning using petroleum ether, ethyl acetate and methanol to obtain three solvent extracts which were subjected to insecticidal test. The petroleum ether fraction (500 mg) was the most active and was chromatographed over silica gel, eluting under pressure with increasing amounts of ethyl acetate in petroleum ether. The eluted fractions were monitored by thin layer chromatography using petroleum ether and ethyl acetate (6:4) as developping solvent. Four fractions were obtained which were subjected to the insecticidal test. The most active fraction 2 (10 ml), was concentrated and further purified by flash chromatography using increasing amount of dichloromethane in petroleum ether as eluting solvent. This led to the isolation of compounds 1 and 2, which were re-crystallized using 20% of petroleum ether in dichloromethane.

Insecticidal test

Insecticidal tests were done using adult beetles (*P. cochleariae)*. The beetles were held on a sticky pad and 1 µg dose of the compounds ware applied to underside using an Arnold micro- applicator. By varying the concentrations of the dose applied from 0.01, 0.1 and 1 % of 1µg/insect, the treatment was done in batches of 10 with 2 batches per concentrations. The treated beetles were kept in plastic Petri-dishes at 20°C, and assessed for mortality after 48 h.

The numbers of dead beetles were recorded and the mean mortality (%) for both 1 and 2 were calculated. LD_{50} values were obtained by data analysis using Polo orbit program.

RESULTS AND DISCUSSION

3-acetylmoraldehyde (Figure 1) and agauriasterone (Figure 2) were isolated as colourless needles, M.P. 274-275°C and 119-120°C, respectively, with positive purple coloration with phosphomolybdic acid as a test for terpenes. The spectroscopic data (IR, NMR and MS) conformed to data of the same compounds isolated by Gregoire and Nyembo (1977) from *A. salicifolia* (Lam).

A 1% concentration of the crude extract from which 3-acetylaldehyde (1) and agauriasterone (2) ware isolated had a mortality of 90% against *P. cochleariae*. Compound 1 and 2 had LD_{50} of 0.04 and 0.15 µg respectively. Their insecticidal activities were closer to the standard Rotenone (3) whose LD_{50} is 0.015 µg, which is used comercially as a broad-spectrum insecticide in fruits, vegetables and for the control of fire ants (Burkill, 1935). Therefore these shows that steroidal triterpenes are rich base for exploiting natural insecticides and compounds 1 and 2 have great potential to be developed as natural product insecticides.

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