



ISOLATION AND IDENTIFICATION OF AN ACARICIDAL GLYCOSIDE FROM *Acacia saligna* LEAVES

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ABSTRACT

Plants have received much attention as sources of biologically active secondary metabolites including pesticides because of their eco-friendly nature. The present study indicated that the ethylacetate extract of the *Acacia saligna* leaves exhibited acaricidal activity against the phytophagous twospotted spider mite (*Tetranychus urticae* Koch).

The chromatographic separation methods led to isolation of a pure compound from *Acacia saligna* leaf ethylacetate extract which exerted acaricidal action against the tested mite with LC₅₀ value of 74.13 mg.l⁻¹ after 48 h. Based on chemical (acid hydrolysis) and spectroscopic (¹H, C¹³-NMR and MS) methods, the isolated compound was identified for the first time from plants as 2-hydroxymethyl-9-hydroxy-9-methyl-undecanyl (1 → 1') (O-α-L-arabinopyranosyl-(1" → 4')β-D-galactopyranoside.

INTRODUCTION

The phytophagous twospotted spider mite, *Tetranychus urticae* Koch (Acari: tetranychidae) is one of the most economically important pest worldwide (Helle and Sabelis, 1985). It is a notorious arthropod pest that affects more than 200 plant species including several significant food and fiber crops and ornamentals, leading to reduction or total loss of yield (Dekeyser and Downer, 1994). Estimates on a world scale suggest that elimination of insect pests would increase crop production by about a third (Van Emden, 1989).

Much of the increase in agricultural productivity over the past half century has been the result of controlling arthropod pests by synthetic chemical pesticides (Duke et al 1993). However, control of such pests has become increasingly difficult because of reduced effectiveness of pesticides caused by emergence of pesticidal resistance in arthropod pests as well as the undesirable effects on non-target organisms, environmental problems and human health hazards (Calmasur et al 2006). Therefore, there is a great need for substituting the synthetic acaricides by other safe alternatives from natural sources, such as plants which represent a valuable source of new compounds for agricultural applications.

Within the last few years, many researches have been reported on botanical acaricides (Sundaram and Sloane, 1995; Ahn et al 1998; Boyd and Alverson, 2000; Radwan et al 2000; Mendki et al 2001; Park et al 2002; Chiasson et al 2001, 2004; Mansour et al 2004; Moussa et al 2005; Rasikari et al 2005 and Calmasur et al 2006). In the current investigation, we reported the acaricidal activity of ethylacetate extract of *Acacia saligna* leaves against twospotted spider mite alongside with the isolation and structure elucidation of the active constituent(s) responsible for this activity.

MATERIALS AND METHODS

Plant material

Leaves of *Acacia saligna* Wendl (Mimosaceae) were locally collected at the flowering stage in April, 2003 from plants growing on the Experimental Farm of the Faculty of Agriculture, Fayoum University. Plant taxonomists in the Botany Depart-

ment, Faculty of Science, Cairo University confirmed the taxonomic identification of the plant species. A voucher specimen was deposited in the herbarium of the Biochemistry Department, Faculty of Agriculture, Fayoum University.

Extraction

The air-dried and ground leaves (1.2 kg) were extracted successively with a series of solvents in order of increasing polarity i.e., petroleum ether 40 – 60 °C (5L), chloroform, (CHCl₃) (5L), ethylacetate (EtOAc) (6L) and methanol (MeOH) (6L) at room temperature (27 °C ± 2). The extracts were evaporated to dryness under reduced pressure to obtain 8.5, 18.3, 12.1 and 60.0 g residues respectively. These residues were evaluated for acaricidal activity against the twospotted spider mite (*Tetranychus urticae*).

Acaricidal activity

The adult females of the twospotted spider mites *Tetranychus urticae* Koch (Acari: Tetranychidae) used in this test were obtained from the Department of Plant Protection, Faculty of Agriculture, Fayoum University.

The acaricidal activities of the extracts and the isolated compound were determined by the method described by Barakat *et al* (1984) as follows:

Disks (2 cm diameter) of castor bean leaves were prepared. Five doses (800, 1000, 1200, 1400, 1600 mg.l⁻¹) of each extract, as well as five doses (50, 100, 200, 300, 400 mg.l⁻¹) of the isolated compound in methanol (100 µl) were applied to the leaf disks. Control leaf disks received 100 µl of methanol only. Solvent was evaporated under fumehood for 2 h then each disk was placed on a wet cotton pad in petri dishes (12 cm diameter) and ten adults females of *T. urticae* were transferred onto the treated and control leaf disks. All treatments were replicated five times for each dosage assay and were maintained at 26 ± 2 °C, and 65 ± 2% relative humidity with 16 h light supplied by a series of fluorescent lamps, then mortality were determined after 24 and 48 h from treatment. Tested mites were considered dead if appendages did not respond after being touched with a camel hair brush.

Data obtained from bioassay was corrected for control mortality by using (Abbott's formula,

1925). LD₅₀ values were determined by Probit analysis (SAS, 1995).

Isolation of the bioactive compound(s)

The bioactive ethylacetate extract was subjected to the isolation of the active component(s) as follows:

Nine grams of the EtOAc residue were subjected to chromatographic column (2.7 cm i.d. X 60 cm) packed with silica gel (200 g, 230-400 mesh, Merck) and eluted with the following series of solvent mixtures of CHCl₃: MeOH: H₂O (95:5:0, 90:10:0, 80:20:0 and 70:30:5, v/v/v for each eluent). Ten fractions of each eluent were collected. The eluates were combined on the basis of similarity of TLC profiles to afford 11 fractions and were then tested for acaricidal activity. The bioactive fraction No.9; eluted with CHCl₃: MeOH: H₂O (80:20:0, v/v/v) between (300-700 ml; 1.78 g residue) was further purified on sephadex LH 20 (20 g) column (1.6 cm id x 60 cm) with MeOH (300 ml) as an eluent. The eluates were combined on the basis of similar TLC profiles to give five fractions designated as (A,B,C,D and E). The most abundant active fraction C (802 mg) eluted between 120-190 ml which contains the major compound was further purified on silica gel (50 g) column (1.5 cm id x 60 cm) using CHCl₃: MeOH(70:30, v/v) as an eluent to give 491 mg of pure compound. The purity of this compound was established by its resolution as a single spot in four different TLC solvent systems.

Analytical thin layer chromatography(TLC)

Analytical TLC was performed on Merck pre-coated silica gel plates(F₂₅₄ thickness 0.25mm) using the following solvent systems:

1. n - Butanol - acetic acid - water (4:1:5, v/v/v) upper layer.
2. Chloroform -methanol - water (70:30:5, v/v/v).
3. Ethylacetate - acetic acid -formic acid -water (100:11:11:27, v/v/v/v).
4. Dichloromethane -methanol - water (50:25:5, v/v/v).
5. Chloroform – acetone (50:6, v/v).
6. Chloroform – methanol (80:20 and 70:30, v/v)

Spots on TLC were detected under short and long UV light (254 nm and 365 nm) and by spraying with concentrated H₂SO₄ followed by heating at 105°C for 5 min. Sugars were detected by spraying with naphthoresorcinol phosphoric acid followed by heating at 105 °C for 10 min.

Structure identification of the isolated compound

The isolated compound was characterized by acid hydrolysis and spectroscopic methods.

Acid hydrolysis

The purified compound was hydrolysed (2mg) by heating with aqueous 10% HCl (2ml) in a sealed tube at 100 °C for 4 hours. The aglycone was extracted with diethylether and analysed by TLC with chloroform – acetone (50:6,v/v). The aqueous layer was neutralized with N,N diocetylamine (10% in CHCl₃). After evaporation to dryness. The sugars were identified by TLC with dichloromethane -methanol - water (50:25:5,v/v/v) by comparison with authentic samples.

Spectroscopic Methods

Nuclear Magnetic Resonance (NMR) Spectroscopy.

¹H and ¹³C NMR spectra were recorded in deuteromethanol (CD₃OD) on a Varion Mercury VXR 300 spectrometer (300 MHz for ¹H and 75 MHz for ¹³C) the chemical shifts (ppm) were related to that of the solvent. The spectroscopic NMR experiments were performed at the Central Laboratory, Faculty of Science, Cairo University.

Mass Spectroscopy (MS).

Mass spectra were recorded on a GCMS. QP 1000 EX Shimadzu Mass spectrometer at 70 e.v. The MS experiments were carried out at Macro-analytical Center, Faculty of Science, Cairo University.

RESULTS AND DISCUSSION

The acaricidal activity of the extracts and the isolated compound of *Acacia saligna* leaves is shown in Table (1). EtOAc and MeOH extracts only showed acaricidal effect against phytophagous twospotted spider mite, *Tetranychus urticae* Koch. It is obvious that EtOAc extract was more potent as acaricidal than MeOH extract after both 24 h and 48 h exposure periods. It is also evident from LC₅₀ values that the acaricidal efficacy was increased with increase of exposure period from 24 h to 48 h as LC₅₀ values decreased by increasing the exposure period from 24 h to 48 h.

Table 1. Acaricidal activity of the extracts and the isolated compound of *Acacia saligna* leaves against twospotted spider mite; *Tetranychus urticae* Koch.

| Extract | LC ₅₀ (mg.1 ⁻¹) | |
|-----------------------|--|-------|
| | 24 h | 48 h |
| Pet.ether | - | - |
| CHCl ₃ | - | - |
| EtOAc | 1000 | 600 |
| MeOH | >1600 | 1000 |
| The isolated compound | 398 | 74.13 |

During the last two decades various plant extracts have been tested as botanical acaricides against the spider mite *Tetranychus urticae* Koch. The most toxic extracts were the acetone extract of *Pinrus malus* and diethyl ether extract of *Piper nigrum*, (LC₅₀ = 1.1 and 39.1 mg/ml, respectively, (Barakat *et al* 1984); the acetone extract of *Datura innoxia* and diethyl ether extract of *Cuminum cyminum*, (LC₅₀= 1.1 and 12.9 mg/ml, respectively, Darwish, 1991); the ethanolic extract of *Duranta ellisla*, (LC₅₀ =500 ppm, Nassar *et al* 1995); the essential oils of *Artemisia absinthium*, (LC₅₀= 0.4 mg/cm², Chiasson *et al* 2001); the aqueous ethanolic extract of *Eucalyptus camaldulensis*. (Mansour *et al* 2004); the methanolic extract of *Plectranthus diversus*, (LC₅₀ = 0.25 %, (Rasikari *et al* 2005).

The most potent EtOAc extract was subjected to the isolation of compound(s) responsible for acaricidal activity. Bioactivity – guided separation of the EtOAc extract of the dried leaves of *Acacia saligna* by using chromatographic methods yielded a chromatographically pure compound(491 mg; 5.46%). This compound exerted an acaricidal activity against adult femals of the twospotted spider mite *Tetranychus urticae* Koch with LC₅₀ values of 398 and 74.13 mg.1⁻¹ after 24 h and 48 h respectively. Thus, this compound was in part responsible for the acaricidal action of the EtOAc extract of the dried leaves of *Acacia saligna*.

There are few published reports on natural substances responsible for the acaricidal activity of plant extracts. For example, dictamine (alkaloid) and seselin (coumarine) compounds for the extract of *Skimmia repens* with LC₅₀=300 and 100ppm respectively (Tanaka *et al* 1985), propanoic acid 2,2-dimethyl(1,1-dimethylethyl) phenyl ester from

Conyzae dioscoridis (Farag et al 1989), β amyryl (Sterol) from *Abrus precatorius* extract (Reda et al 1989), carvacrol and β thujaplicine (Terpenoid) from *Thujopsis dolabrata* (LD₅₀=1.240 and 100 mg/ml respectively, Ahn et al 1998), piperocetadecalinone (alkaloid) from *piper longum* fruits, (LD₅₀=246 ppm, Soo-Park et al 2002), and methyl gallate from *schinus terebinthifolius* LC₅₀=58ppm (Moussa et al 2005).

Several factors such as phenological age of the plant (Jackson and Hay, 1994) percent humidity of the harvested material (Tateo and Riva, 1991), plant part chosen for extraction (Chialva et al 1983), and the mode of extraction (Perez -Souto et al 1992), have been identified as possible sources of variation for the chemical composition and toxicity of the extracts.

The chemical structure of the isolated active compound which was obtained as a colourless, fine powder was characterized by using chemical and spectroscopic methods as follows:

On acid hydrolysis, it gave D-galactose and L-arabinose in the molar ratio of 1:1 as the sugar moiety on TLC by direct comparison with authentic samples. The presence of two sugar moieties in this compound was confirmed by ¹H-NMR spectrum Table (2) due to the appearance of two anomeric protons at δ 4.24 (1 H,d,J = 6.6 Hz) and 4.86 (1H,d,J =3.0 Hz) of galactose and arabinose units which are present in the β -anomeric and α -anomeric forms, respectively. The ¹³C-NMR spectrum of this compound Table (2) showed 24 carbon signals out of which 11 carbons accounted for the sugar moiety α -L-arabinopyranose and β -D-galactopyranose including two anomeric carbons (100.63 and 104.70 ppm), two methylene (δ 62.85 and 67.86 ppm) and seven methines groups (between δ 71.12 and 74.71 ppm). The glycosidation shift of C-4' of galactose in comparing with previously reported data (Hostettmann and Marston, 1995) clearly indicated that arabinose and galactose linked to each other through α (1 \rightarrow 4) linkage. Thus, the sugar moiety in this compound is a disaccharide O- α -L-arabinopyranosyl-(1 \rightarrow 4) β -D-galactopyranose. The remaining 13 carbon atom signals were due to the aglycone moiety and identified as two methyl groups (δ 14.46 and 23.73 ppm), one methine (δ 35.13 ppm), one quaternary carbon (δ 68.76 ppm) and nine methylene groups including oxymethylene (δ 70.09 ppm) and hydroxymethylene group (δ 63.99 ppm).

The aglycone was clearly deduced as 2-hydroxymethyl-9-hydroxyl-9-methyl-1-undecanol from the following proton signals, in the ¹H-NMR spectrum Table (2) as follows:

The presence of six methylene groups (C₃-C₈) attached to each others due two the appearance of proton signals at δ 1.29 (8H,brs C₄ to C₇), 1.61 (2H,brs C₃) and 2.32 ppm (2H,t C₈) (Touati et al 2000 and Biavatti et al 2002). Beside these groups another 3 methylene groups out of which two; oxymethylene (2H,dd,C-1) and hydroxymethyl (2H,3,3, 4.4,d, C-12) attached to methine group (C-2) due to the appearance of proton signal at δ 2.81 (1H,m).

The attachment of both ethyl and methyl groups to the quaternary carbon atom (C-9) due to the appearance of proton signals at δ 0.91 (3H,s), δ 2.07 (2H,q) and 0.98 (3H,t) ascribed to C-13, C-10 and C-11. Also, the quaternary carbon atom (C-9) was attached to methylene group (C-8) due to the appearance of C-7 protons (methylene group) as triplet. Whereas, the methine group (C-2) was attached to the methylene group (C-3) due to the presence of C-2 proton signal as multiple. The presence of oxymethylene group in the aglycone moiety clearly indicated that the aglycone moiety is attached to C-1 of the disaccharide moiety by O-glycosidic linkage.

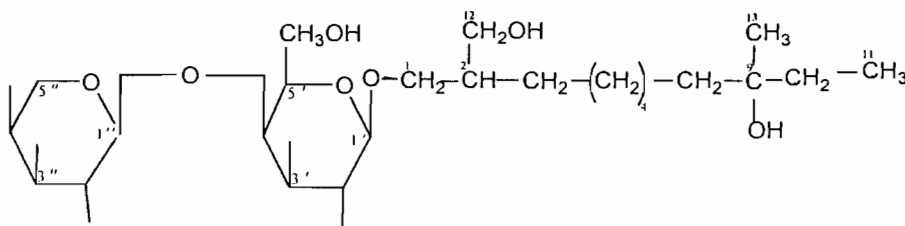
Mass spectral analysis confirmed the structure of this compound since the presence of a molecular peak ion [M]⁺ at m/z 526 corresponding to the molecular formula C₂₄H₄₆O₁₂ along with other diagnostic fragments at m/z 393 (M-C₅H₉O₄; ara), 231 (M- C₁₁H₁₉O₉ (ara+gal); sugar moiety), 195 (Aglycone (C₁₃H₂₇O₃)-36(2H₂O). 167 (C₁₃H₂₃O-28(CHO) and 139 (C₁₂H₂₂ -28 (2CH₂)). On the basis of the above findings this compound Fig. (1) was characterized to be 2-hydroxymethyl-9-hydroxyl-9-methyl-undecanyl (1 \rightarrow 1') O- α -L-arabinopyranosyl (1 \rightarrow 4') β -D-galactopyranoside. This compound was isolated for the first time from this plant.

The literature survey reveals that no prior reports on isolation of this compound, i.e., 2-hydroxymethyl-9-hydroxyl-9-methyl undecanyl (1 \rightarrow 1') O- α -L-arabinopyranosyl-(1 \rightarrow 4')- β -D-galactopyranoside from plants. Thus, this new compound is reported here for the first time in *Acacia saligna* and in the family Mimosaceae. The Long chain alcohol glycoside pattern is rare in nature, however free and esterified alcohols occur widely in nature, e.g., in fruits (Bauer et al 1990).

The isolated acaricidal compound may have potential to be used in sustainable management of *T. urticae*. However, further studies are required to determine the potential of this new compound for control the target arthropod pests under field conditions and for its side effects on nontarget organisms.

Table 2. ^{13}C and ^1H -NMR spectral data of the active isolated compound in CD_3OD

| C- atom No. . | δC | ^{13}C | ^1H |
|------------------|------------------|-----------------|---------------|
| Aglycone | | | |
| 1 | CH_2 | 70.09 | 5.35 dd |
| 2 | CH | 35.13 | 2.81 m |
| 3 | CH_2 | 34.97 | 1.61 brs |
| 4 | CH_2 | 30.78 | 1.29 brs |
| 5 | CH_2 | 30.62 | 1.29 brs |
| 6 | CH_2 | 30.46 | 1.29 brs |
| 7 | CH_2 | 30.20 | 1.29 brs |
| 8 | CH_2 | 33.07 | 2.32 t |
| 9 | C | 68.76 | ----- |
| 10 | CH_2 | 26.40 | 2.07q |
| 11 | CH_3 | 14.46 | 0.98 t |
| 12 | CH_2 | 63.99 | 3.3,4.4 d |
| 13 | CH_3 | 23.73 | 0.91 s |
| Galactose | | | |
| 1 | CH | 100.63 | 4.24 d |
| 2 | CH | 71.50 | 3.70-3.76 m |
| 3 | CH | 72.54 | 3.70-3.76 m |
| 4 | CH | 74.60 | 3.70-3.76 m |
| 5 | CH | 74.71 | 3.70-3.76 m |
| 6 | CH_2 | 62.85 | 3.51 d |
| Arabinose | | | |
| 1 | CH | 104.70 | 4.86 d |
| 2 | CH | 71.79 | 3.87 - 3.90 m |
| 3 | CH | 72.40 | 3.87 - 3.90 m |
| 4 | CH | 71.12 | 3.87 - 3.90 m |
| 5 | CH_2 | 67.82 | 3.49 d |



2-Hydroxymethyl-9-hydroxy-9-methyl-undecanyl (1 → 1')
(O- α -L-arabiopyranosyl- (1'' → 4') β -D-galactopyranoside.

Fig. 1. Structural formula of the active compound

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مجلة اتحاد الجامعات العربية
للعوم الزراعية
جامعة عين شمس ، القاهرة
مجلد (١٧)، عدد (٢)، ٢٩٩-٣٠٦، ٢٠٠٩

الفصل والتعريف الكيميائي لمركب جليكوسيدي ذو الفعليه المبيده للاكاروس من أوراق نبات الأوكاسيا ساليجنا

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١- قسم الكيمياء الحيوية- كلية الزراعة- جامعة الفيوم- مصر

الموجز

وقد أظهر هذا المركب فاعلية ضد أكاروس العنكبوت الاحمر وكانت قيمة LC_{50} هي ٧٤,١٣ ملليجرام/ لتر ، بعد ٤٨ ساعة من المعاملة . وقد تم التعرف علي التركيب الكيميائي لهذا المركب وهو ٢- هيدروكسي ميثيل- ٩- هيدروكسي- ٩- ميثيل- انديكانيل (١-١) الفا- L- أرابينوبرانوسيل (١١-٤) بيتا- D- جلاكتوبرانوسيد ، وذلك باستخدام طرق كيميائية (التحليل المائي بالأحماض) وطرق التحليل الطيفي (طيف الكتلة وطيف السنين المغناطيسي للبروتون والكاربون ١٣).

تزايد الإهتمام بالنباتات في الأعوام القليلة الماضية كبديل للمبيدات المخلفة بما تمثله كمصدر للنواتج الثانوية الفعالة بيولوجيا والتي تستخدم كمبيدات للأفات نظرا لطبيعة هذه المركبات كصديقة للبيئة. وتوضح هذه الدراسة أن مستخلص خلاص الإيثيل لأوراق نبات الأوكاسيا ساليجنا له فاعلية مبيدة ضد أكاروس العنكبوت الاحمر ، وقد أمكن باستخدام طرق التحليل الكروماتوجرافي (العمودي والطبقة الرقيقة) الموجهة بالفاعلية البيولوجية من فصل مركب من هذا المستخلص.

تحكيم: أ.د محمد عبد الرحمن شنتلة
أ.د رضوان صدقي فرج