Secondary Metabolites of the Cuticular Abdominal Glands of Variegated Grasshopper (Zonocerus variegatus L.)

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Chemical compounds were extracted with petroleum ether from the cuticular abdominal glands of grasshopper (Zonocerus variegatus L.) and eleven compounds were characterised using Gas Chromatography/Mass Spectrometry (GC/MS) technique in combination with Fourier Transform-Infrared Spectroscopy (FT-IR). The compounds analysed were 2,7-dimethyloctane (3.21%), decane (5.33%), undecane (3.81%), tridecanoic acid methyl ester (4.76%), hexadecanoic acid (9.37%), 11-octadecenoic acid methyl ester (23.18%), pentadecanoic acid, 14-methyl-methyl ester (4.43%), (Z)-13-docosenoic acid (10.71%), dodecyl pentafluoropropionate (9.52%), 2-dodecyl-1,3-propanediol (6.38%), and 1,12-tridecadiene (19.30%). FT-IR analysis of the extract showed peaks at 1270.17 (C–O and C–F), 1641.48 (C=C), 2937.68 (C–H), and 3430.51 (O–H) cm$^{-1}$ indicating the presence of ether, alkene, alkane, alcohol, carboxylic acid, and fluoric compounds. These compounds consisted of 32.37% ester, 31.65% hydrocarbons, 20.08% fatty acid, 9.52% halogenated ester, and 6.38% alcohol. The highest component was 11-octadecenoic acid methyl ester followed by 1,12-tridecadiene. Since behavioural bioassays were not carried out, the consideration of these compounds to be pheromone semiochemicals remains a hypothesis.

1. Introduction

Chemicals play an important role in communication between insects. Chemicals that mediate interactions between organisms (inter- or intraspecific) are called semiochemicals [1]. The African grasshopper, Zonocerus variegatus (L.) is a tropical insect that belongs to the order Orthoptera and family Pyrgomorphidae. In Nigeria, it usually occurs on uncultivated land with the nymphs and adult stage sharing the same habitat which extends from rain forest zone to the Guinea Savannah in the north [2, 3]. Z. variegatus is a polyphagous insect that causes serious damage to both food and cash crops in West Africa [3–5]. In addition to wild plants it has been recorded as feeding on, and in most cases causing damage to, banana, cassava, Citrus, cocoa, coffee, cola, cotton, cowpea, maize, oil palm, okra, pawpaw, pepper, pineapple, plantain, soybean, teak, rice, various vegetables, and other cash crops such as melon. In southern Nigeria, the staple food, cassava (Manihot esculenta Crantz), is the major crop damaged [6]. Reports from West African countries invariably name this species as one of the major pests against which control measures (generally chemical insecticides) have been applied [7–9]. In 1970, Nigeria declared Z. variegatus a major pest, and subsequently it became a problem in Cote d’Ivoire, Ghana, Congo, Benin, Uganda, Senegal, and Burkina Faso [10].

Chemical insecticides cause or increase environmental pollution and constitute immediate or long-term health hazards. They are danger to human communities, domestic animals, wildlife and plants and they chronically disrupt food-chains. Insecticides eliminate natural enemies which might otherwise keep pest populations within manageable limits [10, 11]. Spraying chemicals on Z. variegatus is only effective in the short-term; hence alternative methods for use by farmers are necessary. It has been reported that both sexes and all instars of the variegated grasshopper, Z. variegatus, when molested, expel an odorous, milky secretions from a gland opening on the dorsal intersegment membrane between the first and second abdominal tergites [12, 13]. It has also been reported that, in studies involving the initiation of
Table 1: FT-IR absorption of the extract from variegated grasshopper (Z. variegatus).

| S/N | FT-IR absorption (cm⁻¹) | Functional group | Nature of compound                      |
|-----|-------------------------|------------------|-----------------------------------------|
| 1   | 1270.17                 | C–O or C–F       | Alcohol, ether, carboxylic acid, ester, fluorocompound |
| 2   | 1641.48                 | C=C              | Alkene                                   |
| 3   | 2937.68                 | C–H              | Alkane                                   |
| 4   | 3430.51                 | O–H              | Alcohol, carboxylic acid                 |

2. Materials and Methods

2.1. Insect Collection. Colonies of Z. variegatus were collected from uncultivated farmland in Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria, and housed in a wire cage (20 × 20 × 30 cm). The organism was identified and authenticated in the Zoology Department of the aforementioned university. About 85 adults of Z. variegatus were used for the investigation and they were maintained on fresh leaves of cassava (M. esculenta) until they were sacrificed for the analysis. Hereafter, the word “grasshopper” refers only to the adults of Z. variegatus unless otherwise stated.

2.2. Extraction of Chemical Constituents. Cuticular abdominal glands of Z. variegatus were excised with fine brand new razor blade after anaesthetising the organisms by cleaning with chloroform which also removed cuticular surface contaminants. Cleaning the insects with chloroform was done with the aid of a piece of foam (a polymeric material) sprinkled with a little amount of chloroform. The tissue was then extracted in petroleum ether for 20 min at room temperature. Extract was placed in screw cap vials and stored at –15°C until analysis.

2.3. Gas Chromatography/Mass Spectrometry (GC/MS) Analysis. GC analysis was carried out in SHIMADZU JAPAN Gas Chromatography 5890-II with a fused GC column (OV-101) coated with polymethyl silicon (0.25 mm × 50 m) and the conditions were as follows: temperature programming from 80–280°C held at 80°C for 1 min, at 200°C for 4 min (rate 10°C/min), and finally at 280°C for 5 min (rate 10°C/min). The injection temperature was 250°C. GC/MS analysis was conducted using GCMS-QP 2010 Plus (Shimadzu, Japan) with column oven temperature of 80°C. The carrier gas was helium with a pressure of 108.2 Kpa and linear velocity of 46.3 cm/s. Total flow was 6.2 mL/min, column flow was 1.58 mL/min, injection mode was split, flow control mode was linear velocity, purge flow was 3.0 mL/min, and split ratio was 1.0. Also, ion source temperature was 230°C, interface temperature was 250°C, solvent cut time was 2.5 min, detector gain was 0.00 KV, detector gain mode was relative, and the threshold was 1000. For the mass spectrometer, start time was 3.0 min, end time was 28.0 min, event time was 0.5 s, scan speed was 1250, and start m/z was 40 while end m/z was 600. The mass spectrometer was also equipped with a computer fed mass spectra data bank. Hermle Z 233 M-Z centrifuge, Germany, was used. All solvents used were of analytical grade and were procured from Merck, Germany.

2.4. Components Identification. The components of the extract were identified by matching the peaks with computer Wiley MS libraries and confirmed by comparing mass spectra of the peaks and those from literature as well as using the database of National Institute of Standards and Technology (NIST) [15].

2.5. FT-IR Analysis. FT-IR measurement of the extract was performed using FTIR-8400S Fourier Transform Infrared Spectrophotometer, Shimadzu, Japan, in a diffused reflectance mode at a resolution of 4 cm⁻¹ in sodium chloride (NaCl) pellets in the range 4500–400 cm⁻¹.

3. Results and Discussion

The chemical constituents of the abdominal glands of Z. variegatus were investigated using GC/MS technique and eleven compounds were characterised as shown by the chromatogram in Figure 1. The compositions of the compounds were 32.37% ester, 31.65% hydrocarbons, 20.08% fatty acid, 9.52% halogenated ester, and 6.38% alcohol. The highest component was 11-octadecenoic acid methyl ester followed by 1,12-tridecadiene. The FT-IR spectra of the extract from variegated grasshopper are shown in Figure 2. FT-IR analysis of the extract showed peaks at 1270.17 (C–O and C–F), 1641.48 (C=C), 2937.68 (C–H), and 3430.51 (O–H) cm⁻¹ indicating the presence of ether, alkene, alkane, alcohol, carboxylic acid, and fluoric compounds. This is summarily shown in Table 1. Table 2 shows the nomenclatures, molecular formulae, molecular weights, retention times, peak areas, and the nature of the analysed compounds. The mass spectra of the eleven compounds are shown in Figures 3–13 while their structures are shown in Figure 14.

This current investigation explicitly shows that variegated grasshopper contained many fractions in the cuticular abdominal regions which constituted esters, alkenes, alkanes, alcohol, carboxylic acids, and fluoric compounds. However, esters and hydrocarbons constituted the bulk of the extract...
Table 2: Compounds identified from the GC-MS analysis of the abdominal extract of variegated grasshopper (*Z. variegatus*).

| Chromatogram peak | Compound name                     | Molecular formula | Molecular weight | Retention time (min) | Peak area (%) | Nature of compound |
|-------------------|-----------------------------------|-------------------|------------------|----------------------|---------------|--------------------|
| 1                 | 2,7-Dimethyloctane                | C_{10}H_{22}       | 142              | 5.703                | 3.21          | Hydrocarbon        |
| 2                 | Decane                            | C_{10}H_{22}       | 142              | 7.078                | 5.33          | Hydrocarbon        |
| 3                 | Undecane                          | C_{11}H_{24}       | 156              | 8.455                | 3.8           | Hydrocarbon        |
| 4                 | Tridecanoic acid methyl ester     | C_{14}H_{30}O_{2}  | 228              | 17.151               | 4.76          | Ester              |
| 5                 | Hexadecanoic acid (palmitic acid) | C_{16}H_{32}O_{2}  | 256              | 18.526               | 9.37          | Fatty acid         |
| 6                 | 11-Octadeconoic acid methyl ester | C_{19}H_{38}O_{2}  | 296              | 20.196               | 23.18         | Ester              |
| 7                 | Pentadecanoic acid, 14-methyl-methyl ester | C_{17}H_{36}O_{2}  | 270              | 20.535               | 4.43          | Ester              |
| 8                 | (Z)-13-Docosanoic acid (erucic acid) | C_{22}H_{40}O_{2}  | 338              | 21.248               | 10.71         | Fatty acid         |
| 9                 | Dodecyl pentafluoropropionate     | C_{15}H_{36}F_{2}O_{2} | 352         | 22.753               | 9.52          | Halogenated ester  |
| 10                | 2-Dodecyl-1,3-propanediol         | C_{15}H_{32}O_{2}  | 244              | 24.145               | 6.38          | Alcohol            |
| 11                | 1,12-Tridecadiene                 | C_{13}H_{26}       | 180              | 24.572               | 19.30         | Hydrocarbon        |
from variegated grasshopper. Some of the identified constituents were fatty acids and alcohols. The fatty acids include palmitic and erucic acids. The hydrocarbon constituents of many insects are synthesized from fatty acids in a series of steps that involve chain shortening or elongation and desaturation and also through modification of the functional group.
by reduction, acetylation, or, sometimes, oxidation [16]. Hydrocarbons have been documented as sex pheromones of some insects; for instance, Peschke [17] reported that cuticular hydrocarbons served as cues for sexual recognition in a rove beetle (Staphylinidae). Also, other hydrocarbons, methylalkanes, and (Z)-9-tricosene have been reported as the sex pheromones in housefly [18, 19]. This investigation provides evidence suggesting that the constituents in variegated grasshopper are multicomponents and the constituents may be synergists to one another. In some other insects like termites, it has been reported that some sex pheromones produced by the reproductive sterna gland are identical to the worker trail pheromones but are secreted in much higher quantities, especially in females whose sternal gland is larger [20]. It is therefore worthy to state that the use of some of these compounds extracted from variegated grasshopper as sex, trail, or aggregating pheromones is a hypothesis. A cooperative and mutual stimulation of these compounds may enable effective communication and behavioural pattern of the insect.

4. Conclusion

The chemical compound extracted from the abdominal glands of variegated grasshopper was analysed with GC/MS and FT-IR techniques which revealed the presence of esters, hydrocarbons, fatty acids, and alcohols. Although behavioural bioassays were not carried out to determine whether these compounds are pheromones, development of the synthetic analogue of the compounds and further
investigating the insect response to them might provide a green alternative method of variegated grasshopper control, leading to increased agricultural produce. Further research is therefore required.

**Conflict of Interests**

The authors declare that there is no conflict of interests regarding the publication of this paper.

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