Short Communications

Volatile constituents of the scent gland reservoir of *Aspavia acuminata*

T.O. Olagbemiro[1]

Department of Chemistry, Bayero University, P.M.B. 3011, Kano (Nigeria), 20 September 1983

Summary. Volatile components of the scent gland complex of the adult bug of *Aspavia acuminata*, an agricultural pest from Nigeria, have been identified by the technique of combined gas chromatograph-mass spectrometry. Of specific interest is the presence in the gland reservoir of hex-2-enal, 4-oxo-hex-2-enal and 4-oxo-oct-2-enal.

Aspavia acuminata (Heteroptera: Pentatomidae) is responsible in Nigeria for severe damage to a wide range of commercially important tropical and subtropical fruits, prominent among these being the seeds of cowpea, bean, beniseed, cotton and guinea corn. Both adult and nymph feed on young shoots, heads, stems and foliage of plants causing yellowing and reduced yields. Comparatively few bugs per tree can cause considerable damage[2]. *Aspavia acuminata* is distributed throughout the Federation of Nigeria.

As part of our program of investigation into possible chemical methods for the control of this noxious pest, a study of the volatile constituents of the metathoracic scent gland complex of the adult of this insect was carried out using the technique of combined gas chromatography-mass spectrometry (GC-MS).

Method. Field-collected specimens of *Aspavia acuminata* from the farmlands of the Institute of Agriculture, Ahmadu Bello University, Zaria, Kaduna State, Nigeria, were transported live by air to Cardiff, and there successfully maintained under laboratory conditions. Gas chromatographic (GC) analysis of a 1-gland test sample from the metathoracic scent reservoir of *Aspavia acuminata* was obtained. The gas chromatograph used was a Varian model 1400 gas chromatograph equipped with a flame ionization detector. The 180 cm glass column (i.d. 2 mm) was packed with OV 225 on 60–80 mesh Gaschrom Q, and was preceeded by a 12-cm-long glass precolumn (i.d. 4 mm). In this case, the entire gland, still suspended in a droplet of saline to prevent collapse, was introduced into the precolumn by the wire coil spoon technique[3]. The chromatograph oven temperature was programmed at 10 °C/min from 80 to 200 °C with nitrogen flow rate at 30 ml/min. Comparison of retention time (R_t) of the sample with that of reference compounds was made by co-injection under similar GC conditions.

The components of the scent glands were identified by combined gas chromatography-mass spectrometry (GC-MS). This was achieved by injecting a 7-gland sample in a minimum of saline into the spectrometer using the solventless wire coil spoon technique as above. The Finnigan 4000 quadrupole mass spectrometer was equipped with a 9610 microprocessor gas chromatograph, INCOS real time data system (32 K core) and Printonix data plotter. The G.C. oven temperature was programmed at 4 °C/min. from 80 to 200 °C. Chemical ionization (CI) with methane as the reagent gas, source temperature 240 °C, and electron impact (EI) with source temperature 260 °C, were employed in the analysis of the scent substances.

Discussion. In general, our analysis of the scent volatiles of *Aspavia acuminata*, which had not been studied previously, reveals some divergencies from the results already reported for other pentatomids. Trans-2-Hexenal, trans-oct-2-enal, hexenal, hexanol, tridecane, dodecane, undecene along with the monoterpene α-terpeneol, have previously been recorded in pentatomids[3–6]. The presence of 4-oxo-hex-2-enal in Aspavia acuminata is particularly striking as this has only been observed in very few pentatomids[7,8]. 4-Oxo-oct-2-enal has previously been reported in *Dysdercus superstitiousus*[9], *Dysdercus intermedius*[10], *Oncopeltus fasciatus*[11] and *Musgraevia sulciventris*[12]. Both ketoaldehydes have been observed to function as defence substances, and their separate occurrence has been observed in most previously studied pentatomids[3,7,8]. That 4-oxo-oct-2-enal and 4-oxo-hex-2-enal occur concomitantly in *Aspavia acuminata* lends credence to their pheromonal roles in these bugs. Tridecane and oct-2-enal. together with hex-2-enal, were found to serve as aggregation as well as defence pheromones in Heteropteras[13,14]. Our field observation of *Aspavia acuminata* recorded their gathering in clusters following scent discharge, particularly when they are disturbed. Probably 4-oxo-oct-2-enal and 4-oxo-hex-2-enal together play a unique role in the defence/alarm mechanism and chemical ecology of *Aspavia acuminata*.

Composition of the metathoracic scent gland of *Aspavia acuminata*

Peak No.	RT	% RA	Identification	Mass spectral data m/z (intensity, %)
1	2.5	4.5	Hex-2-enal	EI 98 (M^+; 15), 97 (10), 83 (50), 69 (40), 55 (35), 41 (100) CI 99 (M + 1; 100), 85 (8)
2	4.3	12.3	Dodecane	EI 170 (M^+; 10), 141 (5), 99 (8), 85 (40), 71 (75), 57 (90), 43 (100) CI 169 (M − 1; 30), 141 (22), 98 (60), 71 (100)
3	6.5	30	Tridecane	EI 184 (M^+; 15), 101 (10), 99 (25), 85 (30), 71 (50), 57 (100), 43 (85) CI 183 (M − 1; 35), 141 (18), 112 (35), 85 (100)
4	7.6	3.5	Oct-2-enal	EI 126 (M^+; 8), 108 (12), 83 (85), 70 (90), 55 (35), 41 (100) CI 127 (M + 1; 55), 109 (100), 85 (45)
5	8.7	23.5	4-oxo-hex-2-enal	EI 112 (M^+; 10), 83 (100), 55 (60), 41 (35) CI 113 (M + 1; 100), 85 (80)
6	9.3	18.4	4-oxo-oct-2-enal	EI 140 (M^+; 5), 125 (15), 111 (75), 98 (100), 83 (62), 55 (40) CI 141 (M + 1; 100), 95 (40), 85 (35)
7	14.5	5.1	Unidentified	EI 152 (M^+; 15), 124 (18), 81 (35), 67 (82), 67 (100), 55 85), 41 (90) CI 153 (M + 1; 5), 125 (100), 83 (80)

RT, retention time (min); % RA, percent relative amount; EI, electron impact; CI, chemical ionization; m/z, mass/charge.

1 The author is grateful to the Bayero University Research and Higher Degrees Committee for financial support, Dr B.W. Staddon for his valuable assistance, Dr D.E. Games for arranging mass spectral facilities.

2 Insect Pests of Nigerian Crops, Research Bulletin, University of Ife/Ministry of Agriculture, Western Nigeria (1968).
3 Staddon, B.W., Everton, I.J., and Games, D.E., Comp. Biochem. Physiol. *62B* (1979) 259.
4 Brennan, B.M., Chang, F., and Mitchell, W.C., Envir. Ent. *6* (1977) 169.
5 Weatherstone, J., Q. Rev. chem. Soc. *21* (1967) 287.
6 Aldrich, J.A., Blum, M.S., Lloyds, J.A., and Fales, H.M., J. chem. Ecol. *4* (1978) 161.
7 Gilby, A.R., and Waterhouse, D.F., Proc. R. Soc. B *162* (1965) 105.
8 Gilby, A.R., and Waterhouse, D.F., Nature *216* (1967) 90.
9 Calam, D.H., and Youdeowei, A., J. Insect Physiol. *14* (1968) 1147.
10 Darooghegh, H., and Olagbemiro, T.O., Experientia *38* (1982) 421.
11 Games, D.E., and Staddon, B.W., J. Insect Physiol. *19* (1973) 1527.
12 McLeod, J.K., Horse, I., Cable, J., Blake J.D., Baker, J.T., and Smith, D., J. Insect. Physiol. *21* (1975) 1219.
13 Smith, R.M., N. Z. Jl Zool. *3* (1964) 375.
14 Ishiwateri, T., Appl. Ent. Zool. *11* (1976) 38.

0014-4754/84/080807-02$1.50 + 0.20/0

Irritant resiniferonol derivatives from Egyptian *Thymelaea hirsuta* L.

A.M. Rizk, F.M. Hammouda, S.E. Ismail[1], M.M. El-Missiry[1] and F.J. Evans

Pharmaceutical Science Laboratory, National Research Centre, Dokki, Cairo (Egypt), and The Department of Pharmacognosy, The School of Pharmacy, University of London, 29–39 Brunswick Square, London, WC1N 1AX (England), 25 October 1983

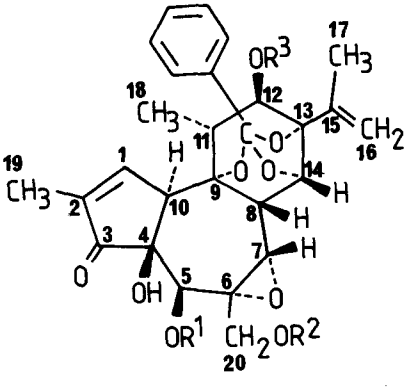
Summary. Two new aromatic derivatives of 5,12-dihydroxy-6,7-epoxy-resiniferonol were isolated from the leaves and twigs of *Thymelea hirsuta* L. (fam. Thymelaeaceae). These compounds were assigned the structures 12-O-cinnamoyl-5-hydroxy-6,7-epoxy-resiniferonol-9,13,14-ortho benzoate **1** and 12-O-heptadecenoyl-5-hydroxy-6,7,-epoxy-resiniferonol-9,13,14-ortho benzoate **2**. Both compounds induced erythema of mouse ears in a dose of 0.1 µg per ear.

The resiniferonol diterpenes are structurally related to the tumor-promoting phorbol-esters from plants of the family Euphorbiaceae[2]. Resiniferonol derivatives were originally isolated from *Daphne* species[3] of the family Thymelaeaceae and are not tumor-promoting agents in 2-stage carcinogenesis tests. However, it has been suggested that they may act at a 3rd stage of carcinogenesis in mammalian systems[4]. Like the phorbol-esters, the resiniferonol-esters induce erythema of skin in low doses[5] but their structure-activity requirements are different, suggesting a different mechanism of action[6].

Thymelaea hirsuta (Thymelaeaceae) is a shrub growing in the Western Egyptian desert and it is known for its toxic activity[7]. 20 kg of dried leaves and twigs were macerated in acetone at room temperature for 1 week. This extract demonstrated pronounced pro-inflammatory activity on the ears of mice using an established assay procedure[5]. After removal of acetone by reduced pressure distillation below 45 °C, the green tar-like residue was dissolved in 40% methanol/water and partitioned against hexane. The hexane fraction contained pigments and lipids and was biologically inactive at doses of 100 µg in the test system above. The methanol/water phase was re-extracted with diethyl ether and the residue from this fraction was shown to induce erythema of mouse ears in a dose of 1 µg per ear. This fraction also exhibited marked cytotoxic activity against TLX/5 mouse lymphoma cells in vitro[8]. The ether fraction was finally freed of green pigment by partition against 0.5% aqueous sodium carbonate solution followed by distilled water until the washings were neutral. Evaporation of the ether solution afforded 15.8 g of a yellow-cream resin. This resin was separated into fractions by means of column chromatography on Florisil using a traditional gradient elution technique[2]. Two biologically active fractions were obtained from the column in yields of about 400 mg.

Fraction 1 was initially purified by means of adsorption preparative-TLC on silica gel G using chloroform/ethyl acetate (2/3) as solvent (R_f 0.10), and further purified by partition preparative-TLC using 20% diethylene glycol on kieselguhr as stationary phase and hexane/butanone (95/5) as mobile phase (R_f 0.22). Final purification was achieved by TLC on silica gel as before using ethyl acetate/acetone (80/20) as solvent (R_f 0.47).

Compound **1** was obtained as a clear glassy resin in a yield of 23 mg. IR ν_{max} (KBr pellet), 3440, 1710, 1660, 1500 cm^{-1}; UV λ_{max} (methanol), 204 ($\varepsilon = 54650$), 215 (shoulder), 280 ($\varepsilon = 42714$), 303 (shoulder) nm; EI-MS (190 °C, 40 e.v., measured values were within 10 ppm of calculated values), $M^{+\cdot}$ m/z 628, $C_{36}H_{36}O_{10}$; CI-MS (190 °C, isobutane), m/z 629 ($M^{+\cdot} + 1$, 50%), 611 (17%), 481 (100%), 419 (7%), 359 (11%), 341 (12%), 323 (11%), 311 (8%), 299 (19%), 253 (20%), 149 (25%), 131 (86%), 123 (43%), 105 (57%); ^{1}H-NMR ($CDCl_3$, 80 MHz, TMS = 0.00 ppm), δ 7.80–7.33 (10H), 7.65 (d, J = 20 Hz, 1H), 7.58 (bs, 1H), 6.37 (d, J = 20 Hz, 1H), 5.18 (s, 1H), 5.05 (s, 2H), 4.95 (d, J = 3.5 Hz, 1H), 4.25 (s, 1H), 3.90 (m,



Compound	R^1	R^2	R^3
1	H	H	OC–CH=CH–⬡
2	H	H	OC–CH=CH–$(CH_2)_{13}CH_3$
3	H	H	H
4	OC–CH_3	OC–CH_3	OC–CH_3