C_9 Aliphatic Aldehydes: Possible Sex Pheromone from Male Tropical West African Shield Bug, *Sphaerocoris annulus*

A. J. E. Gough, D. E. Games, and B. W. Staddon

Departments of Chemistry and Zoology, University College, P.O. Box 78, Cardiff CF1 1XL, U.K.

D. W. Knight

Department of Chemistry, University Park, Nottingham NG7 2RD

and

T. O. Olagbemiro

Department of Chemistry, Abubakar Tafawa Balewa College, Ahmadu Bello University, P.M.B. 0248, Bauchi, Nigeria

Z. Naturforsch. **41c,** 1073–1076 (1986); received June 9, 1986

Sphaerocoris annulus, Heteroptera, Pentatomidae, Scutellerinae, Scent Gland

By gas chromatography-mass spectrometry, ^{1}H NMR, and synthesis of authentic standards, the secretion from the abdominal dorsal scent gland of male adults of *Sphaerocoris annulus* has been found to contain a mixture of C_9 aliphatic aldehydes: nonanal (3%), (*Z*)-4-nonenal (13%), (*E*)-4,8-nonadienal (23%) and (*Z*)-4,8-nonadienal (56%). A further component was tentatively identified as 8-nonenal (5%).

Introduction

Sphaerocoris annulus (F.) is a 1 cm long, distinctively patterned, tropical West African shield bug. Linnavuori [1] places the genus *Sphaerocoris* in the subfamily Scutellerinae within the family Pentatomidae in the Hemiptera-Heteroptera. Concerning the biology of *S. annulus* not a great deal seems to be known. Food plants include species of *Vernonia* (Compositae). The life cycle probably involves an adult reproductive diapause.

It is a peculiarity of the scent gland system of *S. annulus* that the uniquely divided abdominal dorsal first scent gland (DG1) is well developed and biochemically active in the male adults. Only a vestige of DG1 is to be seen in nymphs and female adults. A sex dimorphism in DG1 in the adults in Pentatomidae has been reported previously only in certain Asopinae [2–6] and one other species *(Hotea gambiae)* within the Scutellerinae [7]. In *Podisus maculiventris* (Asopinae) observation has shown that the volatile multicomponent emission from male adult DG1 functions in Nature as a precopulatory aggregation pheromone [6].

Reprint requests to Dr. D. W. Knight.

Verlag der Zeitschrift für Naturforschung, D-7400 Tübingen
0341–0382/86/1100–1073 $ 01.30/0

This report is concerned with the chemical composition of the secretion from adult male DG1 in *S. annulus*. Certain components of the secretion are new to the natural products literature.

Material and Methods

S. annulus adults were collected from several localities in northern Nigeria (Kano, Zaria) during September and October in 1983 and 1984. The host plants on which they were found *(Vernonia pauciflora, V. kotchyana)* were in flower at the time of collection. A few mature nymphs were also collected together with the adults.

Samples of secretion for analysis were obtained from the insects as soon as possible after collection. The opening of DG1 is located on the boundary between abdominal tergites III and IV and can be seen in the adults only after removal of the extensive scutellum (shield) (Fig. 1). The contents of glands excised from chilled adults were extracted in acetone to give a final volume of ca. 2 µl. Samples were stored in sealed glass ampoules at −20 °C. For hydrogenation, ethanolic extracts of DG1 were also prepared, and two samples were prepared in deuterated acetone ($(CD_3)_2CO$) for ^{1}H NMR. For comparison with DG1, acetone extracts were also prepared from the second and third abdominal dorsal scent

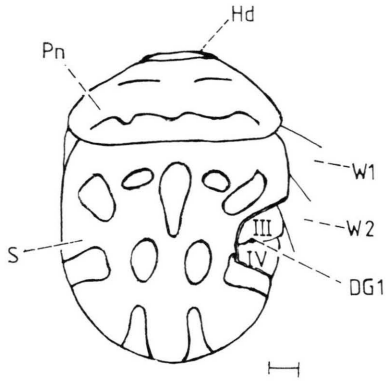

Fig. 1. Dorsal view of adult *Sphaerocoris annulus* showing position of abdominal dorsal scent gland (DG1) beneath the shield (scutellum) towards the right-hand side of the abdomen (III and IV; abdominal tergites three and four). A portion of the scutellum (S) has been removed and the wings (W1, W2) on that same side displaced to reveal the opening from DG1. Topographical details shown include the head (Hd) and pronotum (Pn). Scale line = 1 mm.

glands (DG2, DG3) and from the ventrally situated uniquely adult metathoracic scent gland (MG).

Gas chromatography-mass spectrometry (GC-MS) in the EI mode was performed at 70 eV using according to availability a VG Micromass 7070 E mass spectrometer and a Hewlett-Packard 5970B Mass Selective Detector interfaced to a Hewlett Packard 5890 gas chromatograph. Separations were accomplished on a 30×0.266 mm DB 225 capillary column. The injector was 180 °C, the interface 160 °C, and the oven temperature 70 °C isothermal for 4 minutes and then from 70 to 160 °C at 6 °C/min. The ^{1}H NMR spectrum was recorded at 360 MHz on a WM-360 Bruker instrument. A Varian Vista 6000 gas chromatograph equipped with flame ionization detector was available for peak area comparisons.

Authentic samples of nonanal, (*E*)-2-nonenal and (*E*)-2,6-nonadienal (PPF International) were available for comparison with the *S. annulus* scent volatiles. Other sample materials were prepared as follows:

(Z)-4-nonenal

Alkylation of lithium acetylide-ethylene diamine complex (Aldrich) with 2-(2-bromethyl)-1,3-dioxolane (I) (Lancaster synthesis) in DMSO [8] gave 2-(3-butynyl)-1,3-dioxolane [9] (b.p. 52–54 °C at 14 mm Hg; yield 65%). The acetylene (II) was metallated using *n*-butyl lithium in tetrahydrofuran (−78 → 0 °C) followed by recooling to −78 °C. Sequential addition of hexamethylphosphoramide (1 equivalent) and 1-bromobutane followed by warming to room temperature yielded 2-(3-octynyl)-1,3-dioxolane (III) (yield 87%). Hydrogenation over Lindlar catalyst in chloroform provided the corresponding (*Z*)-alkene (IV) (90%) which was deprotected using 2 M hydrochloric acid in tetrahydrofuran (3 h at 20 °C) to give (*Z*)-4-nonenal (V) (85%) after Kugelrohr distillation; b.p. 80 °C at 12 mm Hg [10].

(E)-4,8-nonadienal

This aldehyde was prepared by oxidation from the corresponding alcohol using pyridinium chlorochromate absorbed on alumina as catalyst [11]. (*E*)-4,8-nonadienal was prepared by coupling 3-butenylmagnesium bromide (synthesis from 3-bromo-1-butene; Aldrich) with 2,3-dichlorotetrahydropyran [12] followed by cleavage of the resulting β-chloro ether using sodium sand [13].

(Z)-4,8-nonadienal

Condensation between 4-pentenal (prep. from 4-pentenol; Aldrich) and the ylide obtained from carboxypropyltriphenylphosphonium chloride [14] using sodium hydride in dimethyl sulphoxide gave 4,8-nonadienoic acid in 58% yield after chromatography. ^{13}C NMR spectra showed the presence of 89% of the (4*Z*) isomer and 11% of the (4*E*). The methyl ester formed from the acid using ethereal diazomethane was reduced by lithium aluminium hydride in ether go give 4,8-nonadienal in 85% yield (b.p. 125 °C at 12 mm Hg). Oxidation of the alcohol by pyridinium chlorochromate gave 4,8-nonadienal in 65% isolated yield (b.p. 80 °C at 12 mm Hg).

Finally, a mixture containing the (*E*)- and (*Z*)-isomers of 4-nonenal was prepared from 1-heptene and acetaldehyde by the method of Nikishin *et al.* [15].

Results

The secretion from male adult DG1 is a highly odoriferous oil. The EI mass spectra of the five peaks revealed by GC and GC-MS (DB 225 capillary column) (Fig. 2) are given in Table I. Ions at $(M-18)^+$ and $(M-29)^+$ in the spectra of all five components indicated a series of aliphatic C_9 aldehydes differing

Table I. Components of secretion from male abdominal dorsal scent gland (DG1) of *Sphaerocoris annulus*.

Peak No.	Peak identity	Composition [%]	EI mass spectrum
1	nonanal	3	142(M^+; 0), 124(2), 114(4), 109(1), 98(17), 96(14), 95(16), 82(25), 70(31), 69(24), 57(87), 41(100)
2	(*Z*)-4-nonenal	14	140(M^+; trace), 122(3), 112(1), 111(2), 98(8), 97(8), 96(15), 84(47), 67(27), 55(58), 41(100)
3	Unidentified	4	140(M^+; 0), 125(0.4), 122(1), 112(3), 111(6), 107(6), 98(16), 97(12), 96(15), 93(16), 81(31), 67(32), 55(100), 41(98)
4	(*E*)-4,8-nonadienal	23	138(M^+; trace), 123(1), 120(3), 110(2), 109(4), 105(3), 97(18), 94(36), 84(18), 79(53), 69(35), 67(57), 55(30), 41(100)
5	(*Z*)-4,8-nonadienal	56	138(M^+; trace), 123(2), 120(3), 110(2), 109(4), 105(3), 97(20), 94(19), 84(22), 79(61), 69(35), 67(56), 55(26), 41(100)

in the degree of unsaturation of the hydrocarbon chain. The identity of peak 1 as nonanal was indicated by computer library search on its mass spectrum and confirmed by comparisons of retention times and mass spectra with authentic *n*-nonanal. The mass spectrum of peak 2 was identified as that of (*Z*)-4-nonenal by comparison of mass spectrum and retention time with authentic (*E*)- and (*Z*)-isomers of 4-nonenal. Peak 3, a minor component (4% total), remains unidentified. Similarities in mass spectrum with peak 2 in the upper mass region are noted. This component could be 8-nonenal but an authentic standard was not available for comparison. The major components (peaks 4 and 5) showed a weak molecular ion at *m/z* 138 and fragments indicating that they were nonadienals. Ammonia chemical ionization gave $(M+1)^+$ ions at *m/z* 139 for both peaks confirming the molecular weight assignment. Diagnostic differences in the mass spectra of these components were looked for but none could be found.

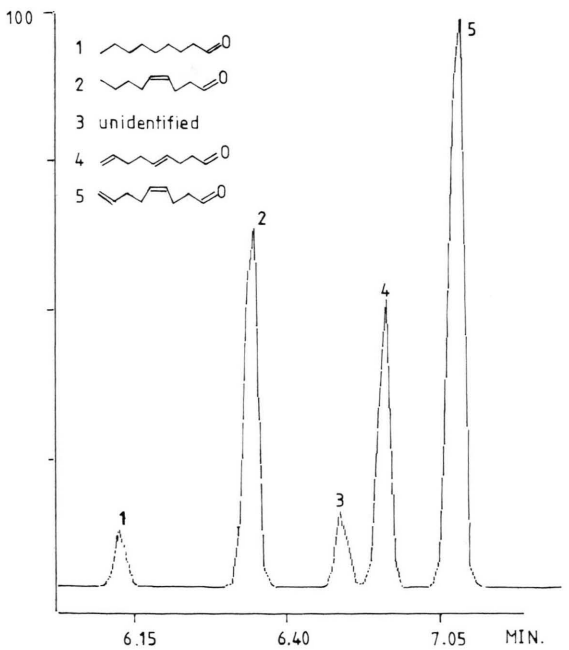

Fig. 2. Reconstructed ion chromatogram from GC-MS of acetone extract of adult male *S. annulus* DG1. Column; 30 m DB 225 capillary. Temperature programme: 70 °C isothermal for 4 minutes and then temperature programme at 6 °C/min to 200 °C.

The ^1H NMR spectrum of the secretion indicated the presence of the (*E*)- and (*Z*)-isomers of 4,8-nonadienal. The resonances observed were triplets at δ 9.91 (0.7 H, *J* 1 Hz) and 9.90 (0.3H, *J* 1 Hz), the aldehydic protons of the (*Z*)- and (*E*)-nonadienals; δ 5.83 (1H complex multiplet), proton on C8; δ 5.40 (1.4H, apparent ABq, *J* 5.5 Hz), protons on Δ4 of (*Z*)-4,8-nonadienal; δ 5.48 (0.6H, apparent ddd, *J* 15, 7, 1 Hz), protons on Δ4 of (*E*)-4,8-nonadienal; δ 4.93 (1H, ddt, *J* 10, 2, 1 Hz) and δ 5.03 (1H, ddt, *J* 17, 2, 2 Hz) for C_9 protons of the terminal olefinic group. The signals obtained for the terminal olefinic group corresponded exactly with those obtained from 1-heptene under the same experimental conditions.

By comparisons of mass spectra and retention times with authentic standards, *Sphaerocoris* DG1 peak 4 was identified as (*E*)-4,8-nonadienal and peak 5 as (*Z*)-4,8-nonadienal.

Hydrogenation of an ethanolic extract of DG1 (10% Pd on carbon; H_2 1 atmos.) yielded as shown by EI-GC-MS almost exclusively nonanal. Reduc-

tion of the C_9 aldehydes to the corresponding alcohols (ethanolic sodium borohydride) was also observed.

Analysis by GC and GC-MS of the secretions from the abdominal dorsal second and third scent glands (DG2, DG3) in mature nymphs yielded the following results: DG2, 2-Hexenal (0.1%), 2-decenal (94%), tridecane (6%); DG3, 2-hexenal (2%), 2-decenal (69%), tridecane (29%). These glands cease to function prior to metamorphosis to the adult. The uniquely adult metathoracic scent gland was found to contain 2-hexenal (25%), 2-octenal (1%), 2-decenal (28%), tridecane (25%), pentadecane (4%), 2-decenyl acetate (8%) and unidentified materials (9%). Unlike DG1, the metathoracic scent gland is morphologically similar in the two sexes. No sex dependent differences in composition in its secretion could be detected.

Discussion

Of the C_9 aldehydes identified in the secretion from adult male *S. annulus* DG1, the (*E*)- and (*Z*)-isomers of 4,8-nonadienal are new, so far as we are aware, to the Natural Products literature. The (*Z*)-isomer is organoleptically distinct from the (*E*)-. Olfactory detection thresholds in both isomers are probably low although not yet established.

Scent gland aldehydes within Hemiptera-Heteroptera as a rule possess even carbon chains. C_6, C_8 and C_{10} species were found in secretions from the other scent glands (DG2, DG3, MG) of *S. annulus*. Only 4-heptenal (C_7) [16] and nonanal [17] have been recorded previously as odd carbon chain aldehydes from the scent glands of Hemiptera-Heteroptera.

The function of *S. annulus* adult male DG1 remains to be elucidated. The volatile emission from male adult DG1 in *Podisus maculiventris* (Pentatomidae-Asopinae) functions as a precopulatory aggregation pheromone [6]. The C_9 scent aldehydes originate perhaps as in cucumber *(Cucumis sativus)* by cleavage from linolenic and linoleic acids [18, 19].

Acknowledgements

We thank the Higher Education Division of the British Council for financial support. M. Rossiter assisted with Mass spectrometry. Purchase of GC-MS equipment was assisted by funding from the SERC; and we thank Hewlett-Packard for the loan of GC-MS equipment.

[1] R. E. Linnavuori, Acta Zool. Fenn. **163,** 1 (1982).
[2] C. Dupuis, C. R. XIII^e Congr. Int. Zool., Paris 1948, 472 (1949).
[3] C. Dupuis, Feuille Nat. **7,** 1 (1952).
[4] C. Dupuis, Cahiers Nat., Bull. **15,** 45 (1959).
[5] J. R. Aldrich, M. S. Blum, H. A. Lloyd, and H. M. Fales, J. Chem. Ecol. **4,** 161 (1978).
[6] J. R. Aldrich, J. P. Kochansky, and C. B. Abrams, Environ. Entomol. **13,** 1031 (1984).
[7] J. G. C. Hamilton, A. J. E. Gough, B. W. Staddon, and D. E. Games, J. Chem. Ecol. **11,** 1399 (1985).
[8] W. N. Smith and F. Beumel Jr., Synthesis **1974,** 441.
[9] R. M. Coates and C. W. Hutchins, J. Org. Chem. **44,** 4742 (1979).
[10] H. J. Bestmann, K. H. Koschatzky, W. Schatske, J. Suss, and O. Vostrowsky, Liebigs Ann. Chem. **1981,** 1705.
[11] Y. S. Cheng, W.-L. Liu, and S.-H. Chen, Synthesis **1980,** 223.
[12] L. Crombie and S. H. Harper, J. Chem. Soc. **1950,** 1707.
[13] M. F. M. Ansell and J. W. Ducker, J. Chem. Soc. **1960,** 5219.
[14] D. B. Denney and L. C. Smith, J. Org. Chem. **27,** 3404 (1962).
[15] G. I. Nikishin, M. G. Vinogradov, and G. P. Il'ina, Synthesis **1972,** 376.
[16] M. S. Blum, Chemical defenses of arthropods, Academic Press, New York 1981.
[17] J. R. Aldrich, W. R. Lusby, J. P. Kochansky, and C. B. Abrams, J. Chem. Ecol. **10,** 561 (1984).
[18] W. Grosch and J. M. Schwartz, Lipids **6,** 351 (1971).
[19] T. Galliard and D. R. Phillips, Biochim. Biophys. Acta **431,** 278 (1976).