J. Insect Physiol., 1975, *Vol.* 21, *pp.* 659 *to* 671. *Pergamon Press. Printed in Great Britain*

UNUSUAL OCCURRENCE OF ALDEHYDES AND KETONES IN THE DEFENSIVE SECRETION OF THE TENEBRIONID BEETLE, *ELEODES BEAMERI*

WALTER R. TSCHINKEL

Department of Biological Science, Florida State University, Tallahassee, Florida 32306, U.S.A.

(*Received* 31 *July* 1974)

Abstract—The defensive secretion of the tenebrionid beetle, *Eleodes beameri*, is quite unlike the benzoquinone and 1-alkene secretion of other species of *Eleodes* and Tenebrionidae. Twenty-three compounds were isolated from the secretion by gas–liquid chromatography (GLC) and 13 of these were identified by infrared, nuclear magnetic resonance, ultraviolet, and mass spectroscopy. Identified compounds were: 1-nonene (3·2%), 1-undecene (<0·5%), *n*-hexanal (15·6%), *n*-heptanal (0·9%), *n*-octanal (4·5%), *trans*-2-hexenal (2·0%), *trans*-2-heptenal (1·5%), *trans*-2-nonenal (28·6%), *trans*-2-decenal (3·4%), *n*-3-nonanone (0·5%), *n*-1-nonen-3-one (16·8%), methyl-1,4-benzoquinone (22·0%), and 1-hexanol (<0·5%). 1-Nonen-3-one is unique to *E. beameri*. A number of minor components remain unidentified. The morphology and ultrastructure of the glands were similar to other species of *Eleodes*. The gland reservoirs are a pair of strongly bilobed sacs with narrow exit ducts opening between abdominal sternites 7 and 8. There are two types of secretory cell units: Type 1 consisting of cells closely attached to the reservoir intima, with large, central vesicles drained by highly convoluted tubules. Type 2 units are composed of a pair of cells functioning together, the cuticular organelle from the microvilli-filled vesicle of the distal cell (2a) passing through the vesicle of the proximal one (2b) and then draining more or less directly into the reservoir via a cuticular tubule.

INTRODUCTION

THE PRESENCE of a pair of abdominal defensive glands consisting of invaginations of the intersegmental membranes between sternites 7 and 8 is so ubiquitous and important a feature among tenebrionid beetles that it has recently been used as a major character for the revision of the family (DOYEN, 1972). The glands and their secretions have been noted in the literature since GISSLER (1879) and WILLISTON (1884) first reported on the defensive habits of *Eleodes* species. Since then, there have been a number of reports on the morphology of the glands (GILSON, 1889; VON LENGERKEN, 1925; ROTH, 1943; PALM, 1946; TSCHINKEL, 1969, 1972; TSENG *et al.*, 1971) and their secretions (see WEATHERSTON and PERCY, 1970, for review) most of which contain benzoquinones and 1-alkenes along with some minor compounds. Exceptions to this pattern were found in *Argoporis alutaceae* Casey which secretes alkylated naphthoquinones (TSCHINKEL, 1972) and *Zophobas*

rugipes Kirsch, which secretes phenols in its prothoracic defensive glands (TSCHINKEL, 1969).

EISNER *et al.* (1964) reported on the ultrastructure of the defensive gland of *Eleodes longicollis* Lec. and described two types of secretory units, each with a large, central vesicle and drained by thin cuticular tubules, and this general pattern was also found by TSCHINKEL (1969, 1972) in *Argoporis* and *Zophobas*.

In the course of a comparative study of the chemical defensive systems of over 150 species of tenebrionid beetles, I found the secretion of *Eleodes beameri* Blais. to be unique through the presence of aldehydes and ketones and the absence of ethylquinone. I report here the isolation and characterization of the components of this complex secretion as well as the morphology and ultrastructure of the gland which secretes them.

MATERIALS AND METHODS

Eleodes beameri Blais. were collected in open coniferous forest at about 8000 ft altitude in the Chiracahua Mountains in southeastern Arizona. They were maintained in boxes of pine and oak litter and fed on wheat bran and scratch grain.

The secretion was collected by handling the beetles, soaking the secretion up on little bits of filter paper, and extracting these in carbon disulphide. The extract was stored in the dark at $-20°C$.

Gas liquid chromatography (GLC)

Analytical traces were run on a Varian Aerograph Model 1400 with a hydrogen flame detector. Columns used were either 5% NPGS or 5% SE-30 (both $\frac{1}{8}$ in. $\times$ 5 ft) on 80/100 mesh Chromosorb W. Nitrogen was used as a carrier gas at flows ranging from 10 to 18 ml/min.

For preparative GLC, a stream splitter set at 1 : 60 was used to pass the bulk of the effluent from a 6% Carbowax 20M ($\frac{1}{4}$ in. $\times$ 5 ft) column to a thermal gradient collector as described by BROWNLEE and SILVERSTEIN (1968). A total of 10 injections was made and 10 fractions were collected in glass capillaries. The capillaries were rinsed with carbon disulphide and each fraction again subjected to preparative GLC on 5% OV-101 ($\frac{1}{4}$ in. $\times$ 5 ft) to obtain the individual compounds. Their purity was checked on 5% OV-101 and 6% Carbowax 20M columns.

Spectroscopy

Nuclear magnetic resonance (NMR) spectra were obtained on a Bruker 90 mHz spectrometer. Infrared (IR) spectra were taken on capillary films on salt chips on a Perkin–Elmer Infracord. Ultraviolet (UV) spectra were taken in hexane solution on a Beckman DB-G spectrophotometer and mass spectra (MS) on an AEI MS-902 mass spectrometer. In addition, the entire secretion was analysed by combined mass spectroscopy–gas liquid chromatography (MS–GLC).

Transmission electron microscopy

Beetles were killed with chloroform vapours and their glands dissected out under

insect Ringer's solution. Preparation for electron microscopy followed the procedure described for *Argoporis alutacea* (TSCHINKEL, 1972) with only minor modifications.

RESULTS

Chemical isolation and characterization

The secretion of *E. beameri* consists of a (usually dark coloured) aqueous phase and a deep yellow organic phase whose odour, unlike that of other *Eleodes* species, is strongly aldehydic. When the beetle is disturbed, it raises its posterior ('head-stands') as do many species of *Eleodes* (EISNER and MEINWALD, 1966; TSCHINKEL, 1975) and allows the secretion to exude from the gland openings at the tip of the abdomen. Usually the seventh sternite is protracted and the pygidium exposed as well.

GLC of the carbon disulphide solution resolved over 23 different compounds. These were isolated by preparative GLC and 9 were obtained in quantities allowing spectroscopy. The spectra indicated the presence of alkenes, alkanals, alkenals, and ketones, and these were identified as below.

TABLE 1—DIAGNOSTIC SPECTRAL DATA FOR ALKENES FROM THE DEFENSIVE SECRETION OF *E. beameri*

	GLC peak No.	
	I	III-A
IR bands (μm)	3·42, 3·33 (C—H) 6·10 (C=C) 10·22, 11·3 (vinyl)	—
NMR (ppm)	1·95 (broad) 4·80–4·90 (complex) 5·50–5·93 (complex)	— —
UV (λ_{max}, nm)	No absorption	—
Mass spectral peaks (m/e)	126 (M^+)	154 (M^+)
Coinjection on GLC	Single peak with 1-nonene on 5% NPGS, 5% SE-30	Single peak with 1-undecene on 5% NPGS
Identity	1-Nonene	1-Undecene
Wt (%)	3·2	Less than 0·5%

A strong C—H stretching band (3·33 μm) and concerted vinyl deformation (10·22 and 11·3 μm) in the IR spectrum indicated that peak I was a 1-alkene (Table 1), and this is in accord with the NMR (3 vinyl protons between 4·80 and 5·93 ppm) and UV data as well. The ratio of methyl to methylene absorption (1 to 3·8) in the NMR and the fragmentation pattern in MS indicated a normal

alkyl group and set the molecular weight at 126, allowing this compound to be identified as *n*-1-nonene. Insufficient amounts of compound III-A were present to allow spectral analysis, but MS–GLC indicated a molecular ion of 154 and fragmentation pattern of *n*-1-undecene. Both these alkenes were confirmed by coinjection with authentic standards on GLC.

Strong IR bands at 3·45 and 5·86 μm and the triplets at 9·65 ppm in the NMR spectra of compounds II and IV indicate that these are aldehydes. The absence of absorption between 5 and 7 ppm in the NMR indicates that they are saturated (Table 2) and this is supported by the lack of strong absorption in the UV. The ratio of methyl to methylene absorption in the NMR is about 1 to 3·7 and points to an unbranched carbon chain. The molecular ions of 100, 114, and 128 and the characteristic fragmentation patterns allow the identification of peaks II, III-B, and IV as *n*-hexanal, *n*-heptanal and *n*-octanal, respectively.

TABLE 2—DIAGNOSTIC SPECTRAL DATA FOR ALKANALS FROM THE DEFENSIVE SECRETION OF *E. beameri*

	GLC peak No.		
	II	III-B	IV
NMR (ppm) ($\mathcal{J}$ in Hz)	1·57 (complex, $\mathcal{J}$ = 8·0) 2·24 (complex, $\mathcal{J}$ = 7·0) 9·65 (triplet, $\mathcal{J}$ = 1·5)	—	1·54 (complex) 2·29 (complex $\mathcal{J}$ = 7·0) 9·65 (triplet, $\mathcal{J}$ = 1·5)
UV (λ_{max}, nm)	No strong absorption	—	—
Mass spectral peaks (*m/e*)	100 (M^+) 99 (—H) 82 (—H_2O) 72 (—CH_2=CH_2) 57 (—CH_2=CHO) 56 (—CH_2=CHOH) 44 (—C_4H_9)	114 (M^+) 113 (—H) 96 (—H_2O) 86 (—CH_2=CH_2) 71 (—CH_2=CHO) 70 (—CH_2=CHOH) 44 (—C_5H_{11})	128 (M^+) 127 (—H) 110 (—H_2O) 100 (—CH_2=CH_2) 85 (—CH_2=CHO) 84 (—CH_2=CHOH) 44 (—C_6H_{13})
Identity	Hexanal	Heptanal	Octanal
Wt (%)	15·6	0·9	4·5

NMR absorption at 9·40 ppm for peaks III-C, VI-A, and VIII again indicated aldehydes (Table 3), but the splitting of this absorption into a doublet rather than a triplet, the absorption between 5 and 7 ppm and the ultraviolet absorption at 220 nm (in hexane) indicated a double bond conjugated with the aldehyde carbonyl. The C-2 proton absorption is split into a double doublet by coupling with the C-3 proton ($\mathcal{J}$ = 15·5 Hz) and the aldehyde proton ($\mathcal{J}$ = 7·5 Hz). The C-3 proton, on the other hand, is split into a doublet by coupling with the C-2 proton ($\mathcal{J}$ = 15·5 Hz) and each of these peaks is again split into a triplet by the adjacent C-4 methylene protons ($\mathcal{J}$ = 7 Hz). The large coupling constant between the proton on C-2 and

TABLE 3—DIAGNOSTIC SPECTRAL DATA FOR ALKENALS FROM THE DEFENSIVE SECRETION OF *E. beameri*

	GLC peak No.			
	III-C	V-A	VI-A	VIII
IR bands (μ)	—	—	3·24 (C—H) 5·90 (C=O) 6·06 (C=C) 10·25 (*trans*-C=C)	3·42 (C—H) 5·91 (C=O) 6·08 (C=C) 10·28 (*trans*-C=C)
NMR (ppm) ($\mathcal{J}$ in Hz)	2·29 (complex) 5·96 (double doublet, $\mathcal{J}$ = 16, 7·0) 6·69 (double triplet, $\mathcal{J}$ = 16, 6·5) 9·40 (doublet, $\mathcal{J}$ = 7·0)	—	2·26 (complex) 5·95 (double doublet, $\mathcal{J}$ = 15·5, 7·5) 6·69 (double triplet, $\mathcal{J}$ = 15·5, 7·0) 9·39 (doublet, $\mathcal{J}$ = 7·5)	2·26 (complex) 5·92 (double doublet, $\mathcal{J}$ = 16, 7·5) 6·67 (double triplet, $\mathcal{J}$ = 16, 7·0) 9·38 (doublet, $\mathcal{J}$ = 7·5)
UV (λ_{max} of K-band, nm)	220	—	220	220
Mass spectral peaks (*m/e*)	98 (M^+) 97 (—H) 83 (—CH_3) 69 (CH_2CH=$CHCHO$) 57 55 (—C_3H_7) 41 (C_3H_5) 29 (CHO)	112 (M^+) 111 (—H) 97 (—CH_3) 83 (—C_2H_5) 69 (CH_2CH=$CHCHO$) 57 55 (—C_4H_9) 41 (C_3H_5) 29 (CHO)	140 (M^+) 139 (—H) 125 111 97 83 70 } —C_1 to C_4 alkyl 69 (CH_2CH=$CHCHO$) 55 (—C_6H_{13}) 43 41 (C_3H_5) 29 (CHO)	M^+ not detected 111 97 83 70 } —C_1 to C_4 alkyl 69 (CH_2CH=$CHCHO$) 57 55 (C_4H_7) 43 41 (C_3H_5) 29 (CHO)
Identity Wt (%)	*n-Trans*-2-hexenal 2·0	*n-Trans*-2-heptenal 1·5	*n-Trans*-2-nonenal 28·6	*n-Trans*-2-decenal 3·4

TABLE 4—DIAGNOSTIC SPECTRAL DATA FOR KETONES FROM THE DEFENSIVE SECRETION OF *E. beameri*

	GLC peak No.		
	V-B	VI-B	IX
IR bands (μm)	—	3·21 (C—H) 5·93 (C=O) 6·18 (C=C) 10·35 (*trans*-C=C) 8·45, 8·85 } dialkyl ketone	—
NMR (ppm) ($\mathcal{J}$ in Hz)	—	2·44 (triplet; $\mathcal{J}$ = 7·0) 5·65 (double doublet, $\mathcal{J}$ = 9·0, 3·2) 6·2 approx. (complex)	1·99, 2·01 } (methyl-H) 6·50, 6·62, 6·64 } (aromatic-H)
UV (λ_{max}, nm)	—	221	245
Mass spectral peaks (*m/e*)	142 (M^+) 113 (—C_2H_5) 85 (—C_4H_9) 72 (—C_5H_{10}) 57 (C_2H_5C=O) 43 41	140 (M^+) 125, 111, 97, 83 } (—C_1 to C_4 alkyl) 70 (CH_2C=$OHCH$=CH_2) 55 (O=CCH=CH_2) 43 41	122 (M^+) 94 (—CO) 82 (—CH_3C≡CH) 68 66 (—2 CO) 54 (O=CCH=CH) 40 39
Identity	3-Nonanone	1-Nonen-3-one	Methyl-1,4-benzoquinone
Wt. (%)	0·5	16·8	22·0

C-3 indicates that the geometric isomerism must be *trans*, and this is supported by the IR band at 10·25 μm. Very fine splitting of the C-2 proton ($\mathcal{J} = 1$ Hz or less) by the C-4 methylene protons is also apparent. The C-4 methylene protons appear as a broad complex peak at 2·26 ppm. The molecular ions and fragmentation patterns allowed the identification of *n-trans*-2-hexenal, *n-trans*-2-heptenal, and *n-trans*-2-nonenal, respectively. A molecular ion was not obtained for peak VIII, but its position in the GLC trace and its MS fragmentation indicated it to be *n-trans*-2-decenal.

Several ketones were isolated from the secretion (Table 4), and the yellow colour, distinctive NMR spectrum, UV absorption, and molecular ion identified peak IX as methyl-1,4-benzoquinone (toluquinone). This was confirmed by coinjection with authentic toluquinone on 5% SE-30 and 5% NPGS. Peak V-B was identified from MS–GLC alone as 3-nonanone. The infrared spectrum of VI-B indicated a carbonyl group (5·93 μm) as well as a large alkyl substituent (3·21 μm). Unsaturation is indicated by the presence of absorption between 5·5 and 6·5 ppm in the NMR and that the double bond is conjugated with the carbonyl group is deduced from the ultraviolet absorption (in hexane) at 221 nm, which also suggests a vinyl group. The nature of the fine splitting in the NMR confirms the vinyl group: a double doublet due to a single proton at about 5·65 ppm ($\mathcal{J} = 9$ Hz for the *trans* coupling, about 3·2 for *cis*), and unsymmetrical absorption by two protons at about 6·2 ppm. The splitting of these protons is similar to that of the vinyl protons in 1-buten-2-one (Sadtler Spectrum 9262M) but not to that of the protons in 3-penten-2-one (Sadtler Spectrum 7126M) in which the double bond is internal. Computer calculation of the expected NMR spectrum of the vinyl protons gave a spectrum that was practically superimposable over that of peak VI-B, and indicated that the unsymmetrical nature of the downfield proton adsorption was due to the superposition of the inner peaks of two doublets centred at 6·10 ppm ($\mathcal{J} = 3{\cdot}2$ Hz) and 6·18 ppm ($\mathcal{J} = 9{\cdot}0$ Hz), respectively. The molecular ion of 140 suggests a nonenone and the base peak at *m/e* 55 due to cleavage between the 3 and 4 carbons, along with a peak of almost equal intensity at *m/e* 70 due to cleavage between the 4 and 5 carbons with hydrogen transfer allows the identification of peak VI-B as *n*-1-nonen-3-one.

A number of tentatively or unidentified compounds were also found: a strong peak at *m/e* 31 (MS–GLC only) indicated a primary alcohol for peak V-C and the base peak at 56 along with an ion resulting from the loss of water (*m/e* 84) allowed identification of *n*-1-hexanol. The MS of authentic 1-hexanol gave a good match for that of peak V-C.

Although MS and NMR spectra were obtained for peak X-C (about 2% of total), the compound was impure and it was not possible to identify it. In addition, at least three acids with retention times longer than most of the identified compounds were detected but not identified.

Gland reservoir morphology

The glands and reservoirs of *E. beameri* are of generally the same type as those of

E. longicollis Lec. (EISNER *et al.*, 1964) and other large species of *Eleodes* (TSCHINKEL, 1975). The large reservoirs lie in the abdominal haemocoel, exit through a narrow pair of ducts between the seventh and eighth abdominal sternites, and consist of a pair of two-chambered sacs (Fig. 1). Elasticity keeps the exit valves closed and

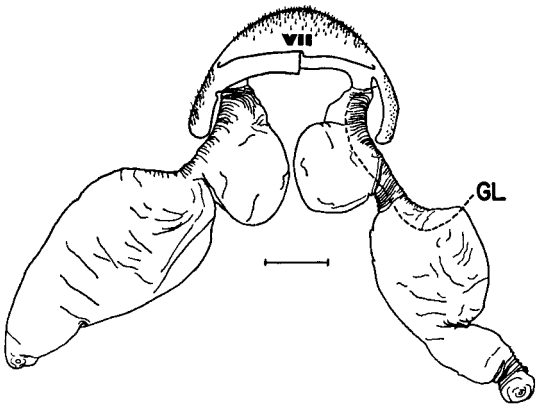

FIG. 1. The gland reservoir sacs after clearing with KOH. VII, Dorsal surface of the seventh abdominal sternite; GL, limits of distribution of the secretory tissue. The bar indicates 1 mm.

they are opened by a muscle inserted on the dorsal side of the exit ducts. The clusters of glandular cells are restricted to the fields indicated by the dotted lines (Fig. 1), but the entire surface of the reservoirs is covered with a thin epidermis *in vivo*.

Ultrastructure of the secretory cells

The secretory cells of *E. beameri* are broadly similar to those of *E. longicollis* (EISNER *et al.*, 1964) and can be similarly classed into two types of secretory units. Cells of Type 1 adhere directly to the epidermis, are very large, have a large central vesicle with lamellar borders (Fig. 3b), and contain a cuticular organelle or 'end apparatus' which is continuous with the tubule draining into the reservoir. These tubules are secreted by a separate cell(s) and are highly convoluted. The outer region of the end apparatus consists of more or less dense cuticle provided with pores while the thicker inner region is fibrous or spongy in appearance. On the haemolymph side the cells are bounded by a basement lamella as well as the plasma membrane which is invaginated into numerous folds or channels extending into the cytoplasm. The cytoplasm is almost completely filled with a smooth, vesicular endoplasmic reticulum (Fig. 3b). Mitochondria containing dense bodies and large, complex membrane-bounded granules are also common, the former near the margins of the vesicle, the latter more distant. Occasional examples of this type of cell appear to have multiple vesicles whose boundaries are more highly lamellar than single-vesicle cells and whose nuclei are highly lobular. It is possible that these represent a phase in the secretory cycle.

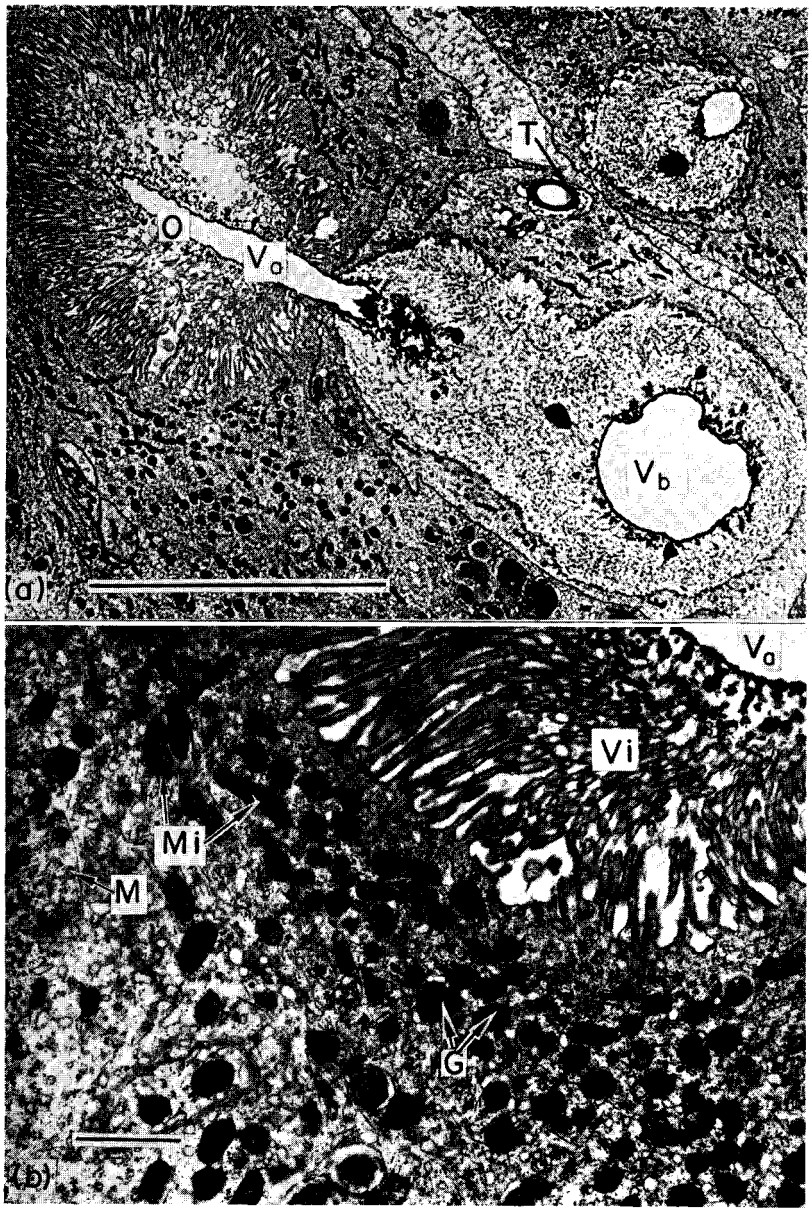

FIG. 2. Electron micrographs of the secretory cells of the defensive glands of *E. beameri*. (a) Type 2a (left) and 2b (right) cells showing the cuticular organelle (O) which originates in the vesicle (V_a) of the 2a cell and passes through the vesicle (V_b) of the 2b cell before draining into the reservoir via a tubule (T). Note the microvilli and abundant mitochondria in the 2a cell. Bar, 10 μm. (b) Higher magnification of the margin of the central vesicle (V_a) of the 2a cell. M, Microtubules; Mi, mitochondria; Vi, microvilli; G, granules. Bar, 1 μm.

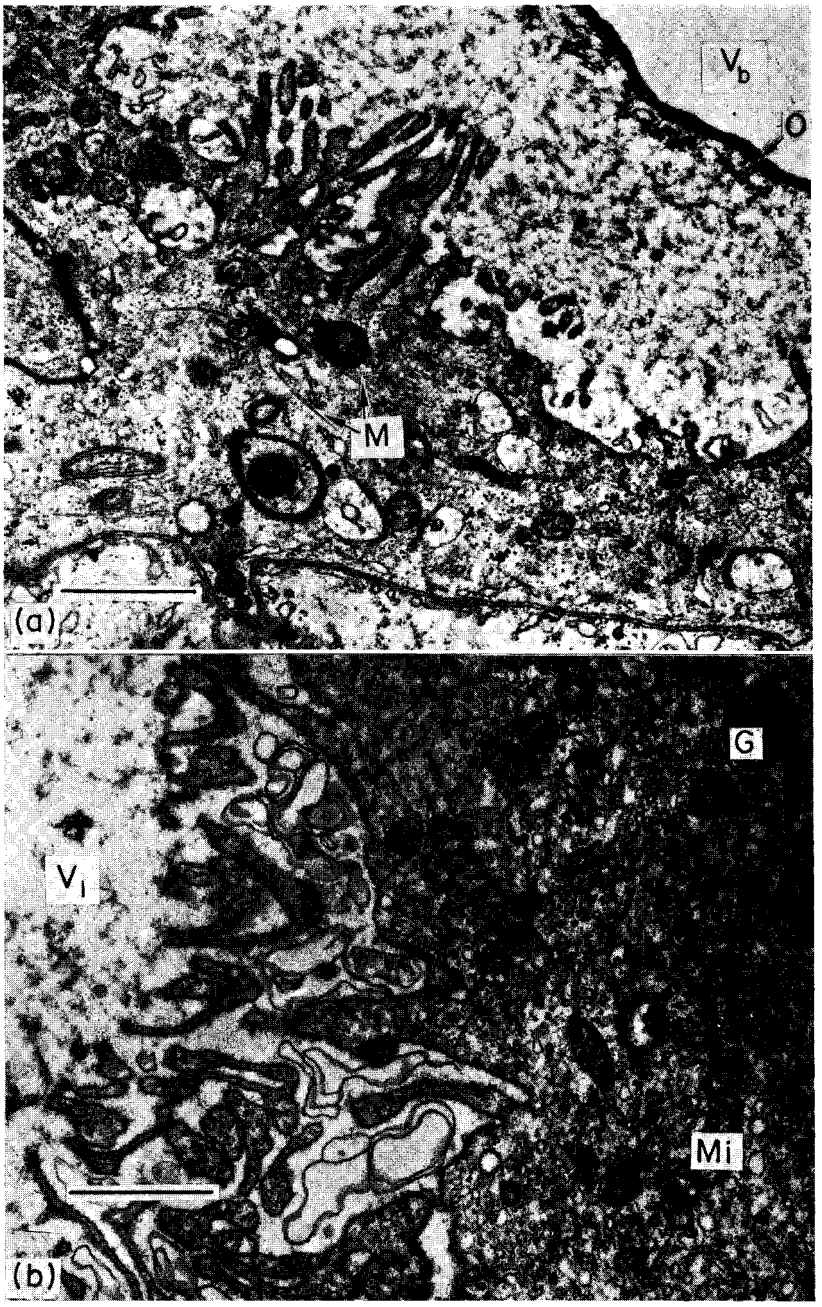

FIG. 3(a). Margin of the central vesicle of the 2b cell. V_b, Central vesicle; M, microtubules; O, cuticular organelle. Bar, 1 μm. (b) Margin of the central vesicle of the type 1 cell. Mi, Mitochondria; G, granule; V_1, vesicles. Bar, 1 μm.

Cells of the 2a and 2b type are always found in pairs (Fig. 2a) and seem to form a functional unit. The porous cuticular organelle which begins in cell 2a, enters cell 2b, and expands into a bulb before exiting as the tubule of the tubule-carrying cell(s) and draining into the reservoirs. The central vesicle of the 2a cell is usually almost filled with microvilli (Figs. 2a, 2b), but these are mostly absent from the vesicle of the 2b cell (Fig. 3a). Endoplasmic reticulum of the 2a cell is smooth and vesicular, there are abundant microtubules, and the numerous and dense mitochondria and membrane-bound homogeneous granules are arranged in a zone surrounding the central vesicle. Occasional examples of what may be exocytosis are visible at the bases of the microvilli. Free ribosomes and glycogen-like granules are fairly common. In the 2b cell, the cuticular organelle has a continuous outer margin and a more conspicuously fibrous inner region (Fig. 3a). The margins of the vesicle are not microvillous, but the cytoplasm is irregularly vesicular and contains numerous free ribosomes. Mitochondria and membrane-bounded granules with membranellar contents are present, but not as abundant as in the 2a cell. The membrane junctions of the 2a and b cells are septate.

The tubules draining the 2a and b cells are much larger in diameter than those draining the Type 1 cell, and they follow a straighter course.

DISCUSSION

The presence of saturated and unsaturated aldehydes and ketones in the secretion of *E. beameri* is highly unusual for the defensive secretions of the genus *Eleodes* as well as the entire family of tenebrionid beetles, most of which secrete benzoquinones and 1-alkenes (SCHILDKNECHT and WEISS, 1960; EISNER and MEINWALD, 1966; TSCHINKEL, 1975). *E. beameri* is further exceptional by the absence of ethylquinone, a ubiquitous compound in all other tenebrionids investigated to date (TSCHINKEL, 1975). Noteworthy also is the presence of such a great number of compounds (over 25), though many are present in small amounts. Among tenebrionid beetles, only the secretion of *Scaurus* with over 50 compounds (TSCHINKEL, 1975) surpasses that of *E. beameri* in complexity. Finally, 1-nonen-3-one is unique to *E. beameri* and has never previously been isolated from any natural source. This is the only investigated species of the subgenus *Holeleodes* and it is possible that other members of this group, whose range barely extends into Arizona from Mexico, share a similar composition.

The compounds may be grouped into several related homologous series implying biosynthetic relationships. All compounds except toluqinone are C_6 to C_{11} alkyl derivatives. Of the functional groups, the most represented are the carbonyl compounds (alkanals, alkenals, alkanones, alkenones, and benzoquinones) totalling about 95 per cent of the secretion, and of the chain lengths, the dominant one is C_9 making up about 50 per cent of the secretion. The second most abundant chain length is the C_6 with about 18 per cent of the total. The alkanal and 2-alkenal series are not completely parallel, nonanal and decanal being absent from the former and 2-octenal from the latter. Among the ketones, the occurrence of 3-nonanone and

1-nonen-3-one may result from shared pathways, and this could also be true of hexanal and hexanol.

Aldehydes and benzoquinones are common components of arthropod defensive secretions and WEATHERSON and PERCY (1970) list 34 species (many Hemiptera) with C_6 to C_{10} 2-alkenals. Saturated *n*-aldehydes, most commonly *n*-hexanal, occur in 18 species and benzoquinones in over 50 species. Recent additions to this list have been made by BRAND *et al.* (1973) and GAMES and STADDON (1973). The secretion of aldehydes by *E. beameri* is thus not unusual for insects, only for tenebrionid beetles.

All the alkanals and 2-alkenals found in insects have been isolated many times from other natural sources, including fish oil, autoxidized milk, frozen peas, carrots, billberries, cranberries, butterfat, cucumbers, cotton buds, and salmon oil (see MINYARD *et al.*, 1967; VON SYDOW and AJOU, 1969; BUTTERY *et al.*, 1971). Interestingly, there are no reports of any vinyl ketones from these or other sources, underlining the uniqueness of 1-nonen-3-one in *E. beameri*.

Ultrastructurally, the defensive gland cells of *E. beameri* resemble those of many other species of insects (NOIROT and QUENNEDEY, 1974) and are especially close to those of other tenebrionids: *E. longicollis* Lec. (EISNER *et al.*, 1964), *A. alutacea* Csy. (TSCHINKEL, 1972) and *Eurynotus capensis* Muls. (K. PRINGLE, unpublished honours thesis). The prothoracic defensive gland of *Z. rugipes* Kirsch. has only one type of cell which seems most closely to resemble the Type 1 cell of *Eleodes*. These four species of tenebrionid differ somewhat in detail: the fine structure of the cytoplasm, the types and abundance of granules, and the degree of microvilli development.

Acknowledgements—I am deeply indebted to HARRIET T. GAGNE' for preparation of the electron micrographs, JIM WHEELER for the combined MS–GLC analysis, and MARTIN SCHWARTZ for his patient help in interpreting the spectra.

REFERENCES

BRAND J. M., BLUM M. S., FALES H. M., and PASTEELS J. M. (1973) The chemistry of the defensive secretion of the beetle, *Drusilla canaliculata*. *J. Insect Physiol.* **19**, 369–382.

BROWNLEE R. G. and SILVERSTEIN R. M. (1968) A micropreparative gas chromatograph and a modified carbon skeleton determinator. *Analyt. Chem.* **40**, 2077–2079.

BUTTERY R. G., SEIFERT R. M., GUADAGNI D. G., and LING L. C. (1971) Characterization of additional volatile components of tomato. *J. Agric. Fd Chem.* **19**, 524–529.

DOYEN J. T. (1972) Familial and subfamilial classification of the Tenebrionoidea (Coleoptera) and a revised generic classification of the Coniontini (Tentyriidae). *Quaest. ent.* **8**, 357–376.

EISNER T., MCHENRY F., and SALPETER M. (1964) Defense mechanisms of arthropods—XV. Morphology of the quinone producing glands of a tenebrionid beetle (*Eleodes longicollis* Lec.). *J. Morph.* **114**, 355–400.

EISNER T. and MEINWALD J. (1966) Defensive secretions of arthropods. *Science, Wash.* **153**, 1341–1350.

GAMES D. E., and STADDON B.W. (1973) Composition of scents from the larva of the milkweed bug, *Oncopeltus fasciatus*. *J. Insect Physiol.* **19**, 1527–1532.

GILSON G. (1889) Les glandes odoriferes du *Blaps mortisaga*. *Cellule* **5**, 3–20.

GISSLER C. G. (1879) Biological notes on some genera of Tenebrionidae. *Bull. Brooklyn ent. Soc.* **2**, 7–8.

VON LENGERKEN H. (1925) Vorstülpbare Stinkapparate der Imago von *Tenebrio molitor L. Biol. Zbl.* **45**, 365–369.

MINYARD J. P., TUMLINSON J. H., THOMPSON A. C., and HEDIN P. A. (1967) Constituents of the cotton bud. The carbonyl compounds. *J. Agric. Fd Chem.* **15**, 517–524.

NOIROT C. and QUENNEDEY A. (1974) Fine structure of insect epidermal glands. *A. Rev. Ent.* **19**, 61–80.

PALM N. B. (1946) Structure and physiology of the stink glands in *Tribolium destructor* Uytt. (Col.). *Opusc. Ent.* **11**, 119–132.

ROTH L. M. (1943) Studies on the gaseous secretion of *Tribolium confusum* Duval—II. The odoriferous glands of *Tribolium confusum. Ann. ent. Soc. Ann.* **36**, 397–424.

SCHILDKNECHT H. and WEISS K. H. (1960) Über die Tenebrioniden-chinone bei lebendem und totem Üntersuchungsmaterial. *Z. Naturf.* **15b**, 757–758.

VON SYDOW E. and ANJOU K. (1969) Aroma of billberries (*Vaccinium myrtillus*)—I. Identification of volatile compounds. *Lebensm. Technol.* **2**, 78–81.

TSCHINKEL W. R. (1969) Phenols and quinones from the defensive secretions of the tenebrionid beetle, *Zophobas rugipes. J. Insect Physiol.* **15**, 191–200.

TSCHINKEL W. R. (1972) 6-Alkyl-1,4-naphthoquinones from the defensive secretion of the tenebrionid beetle, *Argoporis alutacea. J. Insect Physiol.* **18**, 711–722.

TSCHINKEL W. R. (1975) A comparative study of the chemical defensive system of tenebrionid beetles: Chemistry of the secretions. *J. Insect Physiol.* In press.

TSENG Y. L., DAVIDSON J. A., and MENZER R. E. (1971) Morphology and chemistry of the odoriferous gland of the lesser mealworm beetle, *Alphitobius diaperinus* (Coleoptera: Tenebrionidae). *Ann. ent. Soc. Am.* **64**, 425–430.

WEATHERSTON J. and PERCY J. E. (1970) Arthropod defensive secretions. In *Chemicals Controlling Insect Behavior* (Ed. by BEROZA M.). Academic Press, New York.

WILLISTON S. W. (1884) Protective secretions of species of *Eleodes. Psyche* **4**, 168–169.