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6-ALKYL-1,4-NAPHTHOQUINONES FROM THE DEFENSIVE SECRETION OF THE TENEBRIONID BEETLE, *ARGOPORIS ALUTACEA*

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Abstract—The abdominal defensive glands of the western American tenebrionid beetle, *Argoporis alutacea*, contain a viscous mixture of 6-methyl, ethyl-, propyl-, and butyl-1,4-naphthoquinones as well as the usual tenebrionid 1,4-benzoquinones. The naphthoquinones were isolated by preparative gas–liquid chromatography (GLC) and their structures determined by GLC, infra-red, mass, and nuclear magnetic spectroscopy of the isolated compounds and several synthetic isomers. Two additional minor components were not identified.

The tubular, spirally stiffened gland reservoirs are derived from the seventh intersternal membrane and are homologous to other defensive glands in the subfamily Tenebrioninae. Upon handling, the secretion is released by protracting the sixth and seventh sternites and everting the proximal quarter of the reservoir. With its legs, the beetle wipes the secretion onto itself and the handler.

The well-tracheated secretory tissue consists of strands and sheets of cells arranged along and attached by their long effluent ducts which empty along a transverse line near the reservoirs' posterior opening. The surface of the reservoir sacs is covered with a thin epidermis. Electron microscopy of the gland tissue revealed a secretory unit which consists of a long tubular cuticle-lined organelle sequentially ensheathed by three types of cells: (1) a cell with a large microvillus-filled vesicle surrounding the blind distal end of the cuticular organelle, (2) an adjacent second cell into whose central vesicle (which is delimited by small sparse microvilli) the cuticular organelle passes, becoming expanded and diverticulated, and (3) at least one tubule-sheathing cell surrounding each separate cuticular organelle from the point at which it leaves the second cell to its proximal termination at the reservoir.

INTRODUCTION

TENEBRIONID beetles of the subfamily Tenebrioninae, like many other arthropods (EISNER and MEINWALD, 1966) have evolved chemical defensive systems which influence their status as prey for many animals. Among the Tenebrioninae, a wide variety of defensive glands has evolved from a pair of invaginations between the seventh and eighth abdominal sternites, and a number of reports on these glands (LENGERKEN, 1925; ROTH, 1943, 1945; PALM, 1946; EISNER *et al.*, 1964; TSENG *et al.*, 1971) and their secretions have been made. All secretions studied to date have been found to contain *p*-benzoquinones, often mixed with 1-alkenes and fatty acids (SCHILDKNECHT *et al.*, 1964; EISNER and MEINWALD, 1966).

The tenebrionine beetle *Argoporis alutacea* Casey is a conspicuous exception within its subfamily, for its defensive secretion consists largely of 6-alkylated naphthoquinones in addition to the usual benzoquinones. This is the first report of the isolation of this class of compounds from the animal kingdom.

MATERIALS AND METHODS

Argoporis alutacea were collected under stones and debris near Cerro Pinacate, Sonora, Mexico in December of 1966. Although nothing is known of their habits, in the laboratory the adults oviposited well in damp soil, and both the larvae and adults could be reared on wheat bran. Crowded larvae will not pupate, so that when larvae attained their full weight (TSCHINKEL and WILLSON, 1971), they were isolated, whereupon they pupated within about 2 weeks. The emerging adults were allowed at least 2 weeks before they were milked of their secretion. Approximately 300 milkings made up the total sample which was stored in carbon disulphide at −20°C.

Gas–liquid chromatography (GLC)

Analytical traces were run on a Varian Aerograph Model 1200 with a hydrogen flame detector. Columns were either 5% SE-30 ($\frac{1}{8}$ in. × 6 ft) on Chromosorb W 80/100 mesh, or 5% NPGS ($\frac{1}{8}$ in. × 6 ft) on Chromosorb W 80/100 mesh. The carrier gas was nitrogen in all cases.

For preparative GLC, a micro-preparative splitter was set at either 15 : 1 or 30 : 1. The mixture was injected on a 5% SE-30 ($\frac{1}{4}$ in. × 5 ft) column at 140°C and after elution of the benzoquinones, the temperature was programmed to 200°C at 4°C/min. The peaks were collected in glass tubes at room temperature and the condensate was rinsed out with carbon disulphide for rechromatography or NMR spectroscopy.

Spectroscopy

NMR spectra were taken either on a Varian A-60 or a 60 Mc Perkin–Elmer. Infra-red spectra were taken as KBr pellets on a Perkin–Elmer Infracord. Mass spectra were taken on an AEI MS-902 mass spectrometer.

Chemical synthesis

The alkylated naphthoquinones were synthesized according to the procedure of BENDZ (1951) for 6-methyl-1,4-naphthoquinone. Identical procedure resulted in good yields of all isomers synthesized.

Transmission electron microscopy

Beetles were killed with chloroform vapours, their glands dissected out under insect Ringer's solution and fixed in a modified KARNOVSKY (1965) solution containing 1% formaldehyde, 3% glutaraldehyde, and 0·05% $CaCl_2$ in 0·1 M sodium cacodylate (pH 7·4). Glands were fixed (at room temperature) for a total of 3 hr during the first 45 min of which hydrogen peroxide in the amount of 9 drops of

30% H_2O_2/25 ml of fixative was added (C. Franzine-Armstrong, personal communication to Harriet Gagne). The tissue was then prepared and sectioned by the usual procedures.

RESULTS

Chemical isolation, characterization, and synthesis

The odour of *Argoporis*' thick, sticky defensive secretion is reminiscent of freshly sawn Brazilian rosewood. The total secretion consists of two phases: a denser, colourless aqueous phase and a deep orange organic phase. Normally, only the organic phase is released, but after prolonged or very rough stimulation, the aqueous phase may also be expended.

Although GLC of the organic phase showed *p*-benzoquinone, *p*-toluquinone, and *p*-ethylquinone to be present as they are in most species of the subfamily Tenebrioninae, there were also present at least six major peaks with much greater retention times and temperatures than the benzoquinones (Fig. 1). Collection of

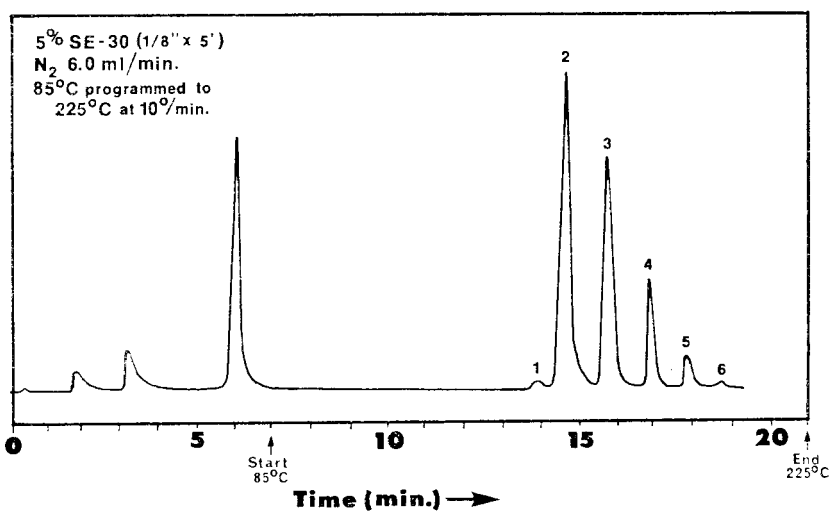

FIG. 1. Gas chromatographic trace of the secretion of *A. alutacea* Casey. The naphthoquinones and associated peaks (1 through 6) elute at much higher temperatures than the *p*-benzoquinones.

these peaks from preparative GLC revealed that they had the yellow colour and sharp odour typical of many quinones. Since they elute so close together, it was necessary to rechromatograph each peak to obtain at least 98 per cent purity. Yields from the total combined secretions were as follows for peaks 1 through 6, respectively: 0·3, 3·6, 1·4, 0·4, 0·3, and 0·2 mg. The purity of each isolate was checked by running a small sample on a 3% Carbowax 20M column ($\frac{1}{8}$ in. × 5 ft; N_2 flow 8·3 ml/min).

The i.r. spectra of peaks 2 and 3 (Table 1) exhibited very strong bands at 6·00 μm indicative of conjugated carbonyls such as in quinones. The NMR spectra of these two peaks (Table 2 and Fig. 2), in addition to the vinyl proton singlet at about 6·93, revealed four broad resonances centred around 7·8 ppm

TABLE 1—INFRA-RED ABSORPTION DATA

Compound	Bands, in order of decreasing intensity
Argoporis peak 2	6·00, 12·19, 6·24, 7·70, 7·54, 9·45, 9·55, 8·81
6-Methylnaphthoquinone	6·00, 12·20, 6·24, 7·70, 7·55, 9·58, 9·48, 8·78–8·90
5-Methylnaphthoquinone	6·00, 12·91, 7·55, 7·78, 11·72, 9·14, 6·30, 6·20, 9·70
Argoporis peak 3	6·00, 7·71, 6·24, 12·00, 7·57, 9·62, 8·50–8·90
6-Ethylnaphthoquinone	6·00, 7·71, 6·25, 12·00, 7·55, 9·60, 8·88, 8·75 (shoulder)
5-Ethylnaphthoquinone	6·02, 7·76, 7·63, 9·12, 11·71, 12·75, 9·69, 6·20, 6·31, 12·00, 8·67, 13·28
Argoporis peak 4	6·03, 7·74, 6·27, 7·90 (shoulder), 7·55 (shoulder), 12·00, 11·15

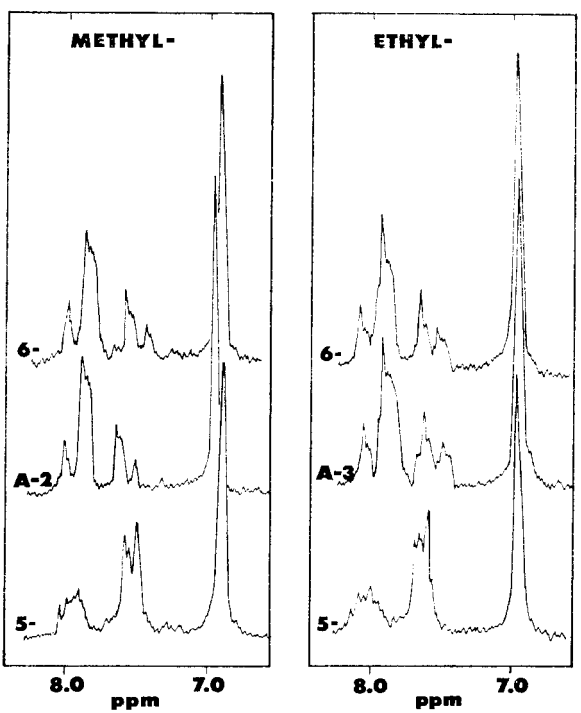

FIG. 2. NMR spectra of the aromatic and vinyl proton regions of *Argoporis* peaks 2 (A-2), and 3 (A-3), and the 5 (5-) and 6-methyl (6-) and ethyl (5-, 6-) of naphthoquinone. In both the correspondence is between the 6 isomer and the natural product.

TABLE 2—NUCLEAR MAGNETIC RESONANCE DATA

Compound	Absorption (ppm)			
	Methyl H*	Methylene H	Vinyl H	Aromatic H†
Argoporis Peak 2	2·63 (singlet)		6·95 (singlet)	7·55, 7·68, 7·92, 8·06
6-Methylnaphthoquinone	2·45 (singlet)		6·92 (singlet)	7·45, 7·60, 7·89, 8·03
5-Methylnaphthoquinone	2·69 (singlet)		6·88 (singlet)	7·55, 8·00
Argoporis Peak 3	1·45 (triplet) $\mathcal{J} = 0{\cdot}13$	2·78 (quartet) $\mathcal{J} = 0{\cdot}12$	6·95 (singlet)	7·55, 7·68, 7·93, 8·08
6-Ethylnaphthoquinone	1·27 (triplet) $\mathcal{J} = 0{\cdot}13$	2·75 (quartet) $\mathcal{J} = 0{\cdot}12$	6·95 (singlet)	7·54, 7·65, 7·94, 8·10
5-Ethylnaphthoquinone	1·29 (triplet) $\mathcal{J} = 0{\cdot}13$	3·22 (quartet) $\mathcal{J} = 0{\cdot}13$	6·97 (singlet)	7·68, 8·08

* The exact position of this peak is concentration dependent. This is true to a lesser degree for the other absorptions also.

† See Fig. 2 for comparison of the aromatic proton absorption of the 5- and 6-substituted compounds.

typical of aromatic protons. Also indicated were methyl protons (singlet, 2·45 ppm) in peak 2 and both methyl (triplet, 1·41 ppm) and methylene (quartet, approximately 2·6 ppm) protons coupled to each other in peak 3. Mass spectroscopy (Table 3) indicated a molecular weight of 172 for peak 2 with each later peak increasing 14 *m/e* units (=CH_2) over each previous peak (peaks 3, 4, and 5).

The evidence pointed to a homologous series of alkylated 1,4-naphthoquinones, beginning with a methylnaphthoquinone (peak 2) and ending with the equivalent of a butylnaphthoquinone (peak 5). The position and isomerism of the substituents remained to be determined. Both NMR and mass spectroscopy, as well as co-injection GLC, were incompatible with substitution on the quinone ring, but

TABLE 3—MASS SPECTRA OF *Argoporis* NAPHTHOQUINONES: DIAGNOSTIC PEAKS

Ion	Peak 2		Peak 3		Peak 4		Peak 5	
	m/e	%	*m/e*	%	*m/e*	%	*m/e*	%
M^+	172	100	186	100	200	100	214	100
M—CH_3	157	6	171	21	185	6	199	10
M—CO	144	21	158	10	172	3	186	18
M—C_2H_5			157	4	171	20	185	6
M—CO—CH_3 or M—C_3H_7			143	8	157	11	171	11
M—C_3H_2O	118	29	132	7				
M—2CO	116	17	130	4	144	2	158	1
M—C_4H_9							157	8
M—C_3H_2O—CO	90	11	104	3	118	6	132	6

indicated substitution on the benzenoid ring. Mass spectra showed peaks resulting from the rupture of the 1,2 and 4,10 bonds (*m/e* 118 for peak 2, 132 for peak 3) and further loss of CO to yield an ion consisting of the substituted benzene nucleus (*m/e* 90 for peak 2, 104 for peak 3) (BUDZIKIEWICZ *et al.*, 1967).

Substitution on the benzene nucleus could be either on the 5 or 6 position, and NMR evidence from related compounds favoured the 6 isomers. Both the 5- and 6-methyl and 5- and 6-ethylnaphthoquinones were therefore synthesized from the appropriate alkyl-1,3-butadienes and benzoquinone by Diels–Alder addition followed by isomerization and oxidation of the products to the corresponding alkyl naphthoquinones (BENDZ, 1951) which crystallized from heptane as beautiful orange needles. The orange colour is due to an impurity and sublimation or chromatography on Florisil yielded bright yellow crystals.

The NMR, i.r., and mass spectra of these synthetic compounds showed congruency with the spectra of 6-methylnaphthoquinone and peak 2, and 6-ethylnaphthoquinone and peak 3 (Tables 1–3, and Fig. 2), and the identity of these compounds is considered as established.

GLC had not yielded enough material from peaks 1, 4, 5, and 6 for NMR, but mass spectra of all these were obtained. Since the molecular weights of peaks 4 and 5 correspond to the propyl and butyl derivatives of naphthoquinone, it was judged that, from biosynthetic considerations, the most likely isomers would be the 6-*n*-propyl and 6-*n*-butyl naphthoquinones. Confirmation awaits the isolation of more material and the synthesis of the candidate compounds.

The parent ions of the mass spectra of peaks 1 (*m/e* 152; naphthoquinone 158) and 6 (*m/e* 202; pentyl naphthoquinone 228) indicated that they were not members of the same homologous series. Though the molecular weight of peak 6 allows the possibility of ethyl methoxynaphthoquinone, its identity and that of peak 1 could not be determined due to the extremely small quantities present. During preparative GLC, it was noticed that the distinctive rosewood-like odour appeared to be due to peak 1. The identities of these compounds are currently under investigation.

DEFENSIVE BEHAVIOUR

The primary function of the secretion is probably as a deterrent to predators. Thus, when the beetles are handled, they give off the secretion by protracting the terminal visible abdominal sternite (sternite VII) ventrally and everting the proximal quarter or so of the reservoir sacs. This exposes a broad fold of chitinous membrane where the secretion collects as a viscous mass. Presumably, it is not stored in this fold, but rather in the gland reservoirs from which it is forced by haemolymph pressure at the appropriate time. If the secretion is not lost from the exposed fold, the beetle is able to withdraw it once more into the reservoirs.

The effectiveness of the secretion is probably due to the general irritating effect of quinones on sensitive tissue (EISNER and MEINWALD, 1966) both by vapours and the liquid. The beetle increases this effectiveness by deliberately spreading the secretion over its own body and that of the attacking animal. When the

secretion is exposed at the end of the abdomen, the beetle wipes its hind tibiae through the viscous fluid and then wipes this fluid onto the finger of the person holding the beetle. Presumably, it would similarly wipe the fluid onto any predator in whose grasp it might find itself. Since many animals hold their prey in their mouths, their sensitive mucous membranes would be particularly vulnerable to irritation. If the first two pairs of legs are not being restrained, the beetle will also wipe the secretion from the hind tibiae into the mesotibiae (or tarsi), from there onto the protibiae (or tarsi) and from there onto the dorsal surface of its head and the bases of its antennae. Thus, no matter where the predator grasps the beetle, it is likely to encounter the repellent secretion. This type of 'spreading about' of defensive secretion is common among tenebrionid beetles and has probably evolved independently many times (Tschinkel, in preparation).

In addition, the beetle spreads its legs and stiffens them, raising the posterior slightly. The significance of this response is unknown, but it is common in many tenebrionids both with and without defensive glands, and is probably the 'pre-adapted' motor pattern which gave rise to the conspicuous 'headstanding' behaviour in *Eleodes* (EISNER and MEINWALD, 1966) and related genera (Tschinkel, in preparation).

GLAND MORPHOLOGY AND ULTRASTRUCTURE

Gross structure

The reservoir sacs are invaginations of the intersegmental membrane between the seventh and eighth sternites and project forward about half the length of the abdominal haemocoel (Fig. 3) (the seventh sternite is the last visible one and must be protracted to extrude either the genitalia or the defensive glands). The reservoirs are tubular, tapering toward their blind distal ends and having some common volume at their proximal end. The intima of the sac walls is somewhat stiffened by spiral thickenings of folds which allows accordion-like extension as the sac fills. These thickenings are very developed as prominent ribbing (R) in the proximal lateral regions of both sacs. These latter cuticular bands probably are related to the partial eversion of the sacs, perhaps preventing them from being everted beyond a certain point. Through the semi-transparent walls, the secretion can readily be seen to consist of an orange organic phase and a colourless aqueous phase. Just dorsal to the sac openings is a fold of cuticle which, when retracted, forms a pocket, but when extruded, forms a broad surface over which the secretion spreads. The dorsal side of the seventh sternite forms a platform onto which the secretion also spreads.

The gland cells are found in sheets and strands arranged along and attached by their effluent ducts which join the sac wall along a transverse line near its exterior opening. The duct of each secretory unit runs the full distance to the junction with the sac and the ducts of 30 to 40 cells are collected into bundles. Near the point of attachment to the sac, the ducts in the bundle undergo some fusion, beginning in the centre and progressing toward the perimeter as the sac is approached, so that short hollow cones project out from the sac wall where each

bundle merges with it. After digestion with KOH, the ducts appear as very fine hair-like strands, often iridescent green due to interference.

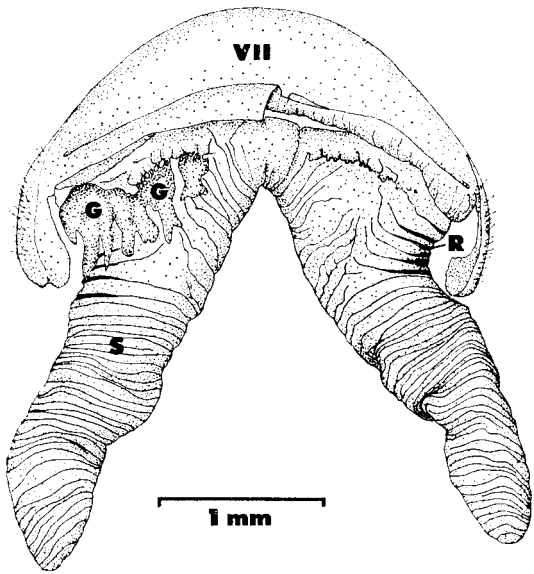

FIG. 3. Dorsal view of the defensive glands of *Argoporis* after digestion in KOH. The glandular tissue (G) has been restored on the left side and entirely removed on the right. *In vivo*, the entire surface of the reservoirs is covered with a thin epidermis, but the gland tissue is present as strands and sheets attached only by their effluent ducts. Thickened cuticular ribbing (R) occurs on the outside of both reservoirs and probably represents the normal limit of eversion. The reservoir sacs (S) are attached to sternite 7 whose dorsal surface is indicated by VII.

Fine structure

A secretory unit consists of three cells: one type 2a cell, one type 2b cell, and at least one tubule-carrying cell (nomenclature that of EISNER *et al.*, 1964). The appearance of each is described below.

The type 2a cell (Figs. 4, 5) is large and contains a highly vesiculated cytoplasm, a large central region into which many long microvilli intrude, and a nucleus displaced to one side of this central vesicle. The microvilli contain fine filamentous cytoplasm and arise from a distinct region of the cell where the cytoplasm has a similar appearance and a lack of defined organelles. At their distal ends the microvilli surround a blind, hollow cuticular organelle whose walls at high magnification are seen to have a porous, honeycomb configuration. The frothy appearance of the cytoplasm is attributable mostly to small smooth-surfaced vesicles, perhaps smooth reticulum, and to mitochondria which are characteristically somewhat inflated. It appears as if some of the cytoplasmic vesicles empty into the central cavity, at the base of the microvilli. Scattered lysosomes are consistently seen in the cytoplasm and microtubules occur near the cell border, but other discrete

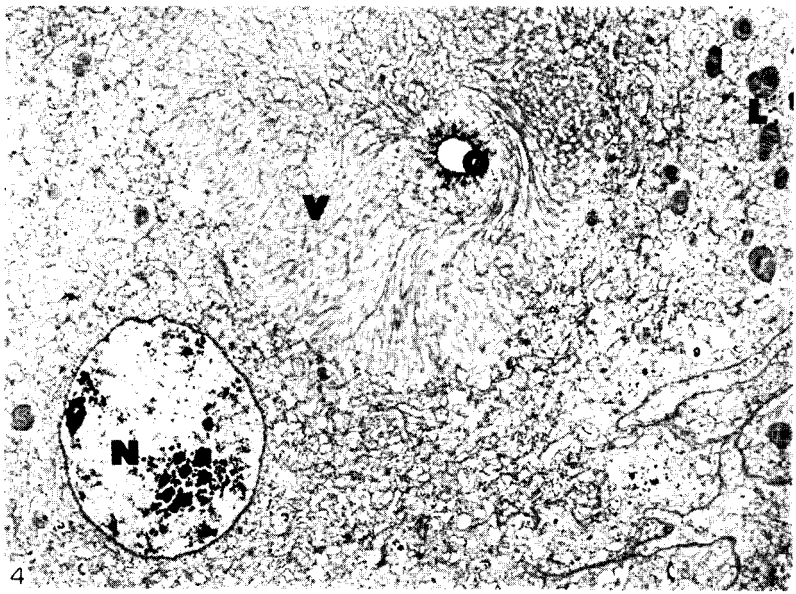

FIG. 4. Electron micrograph of a portion of the 2a cell showing the microvillus-filled central vesicle (V) with its cuticular organelle (O). Note the 'frothy' cytoplasm and the lysosomes (L).

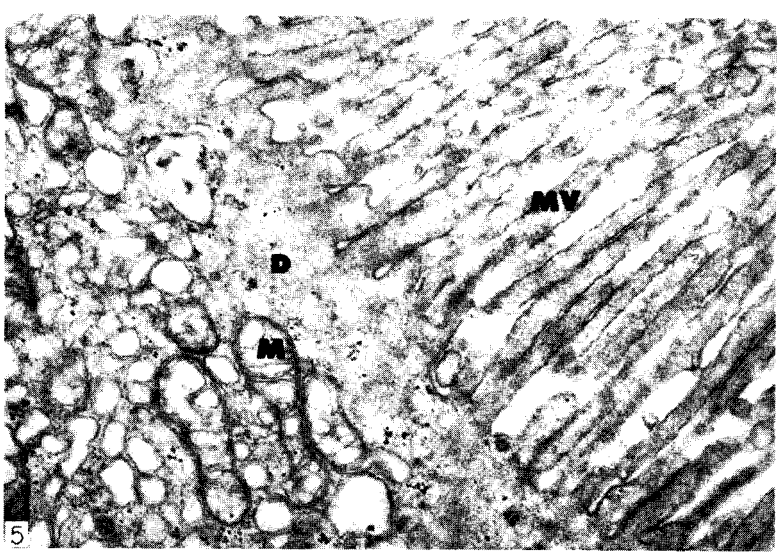

FIG. 5. Higher power electron micrograph of the edge of the central vesicle of the 2a cell. Note the microvilli (MV), the mitochondria (M), and the region subjacent to the base of the microvilli consistently devoid of organelles (D).

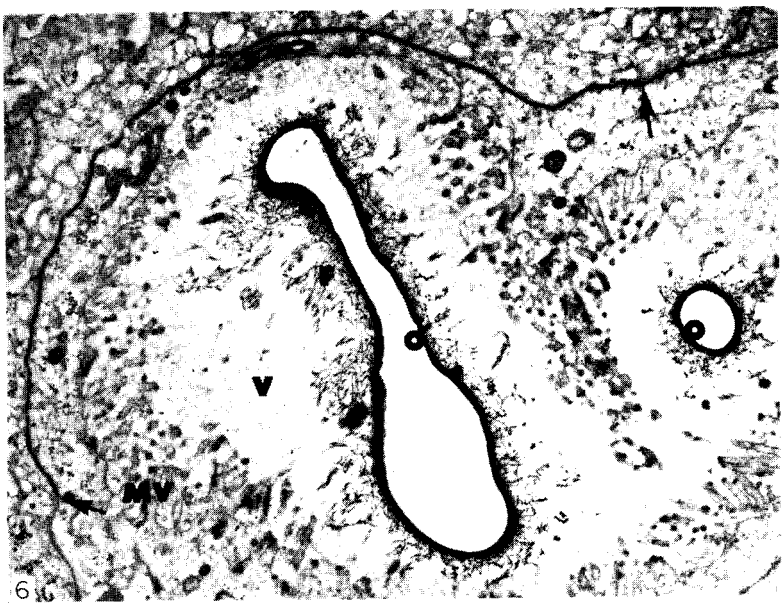

FIG. 6. Electron micrograph of the 2b cell with its cuticular organelle (O) (here cut in two places) surrounded by filaments (F). Short microvilli (MV) project into the vesicle (V) in which the cuticular organelle lies. The membrane between the arrows appears to be a septate portion of the shared boundary between the 2a and 2b cells.

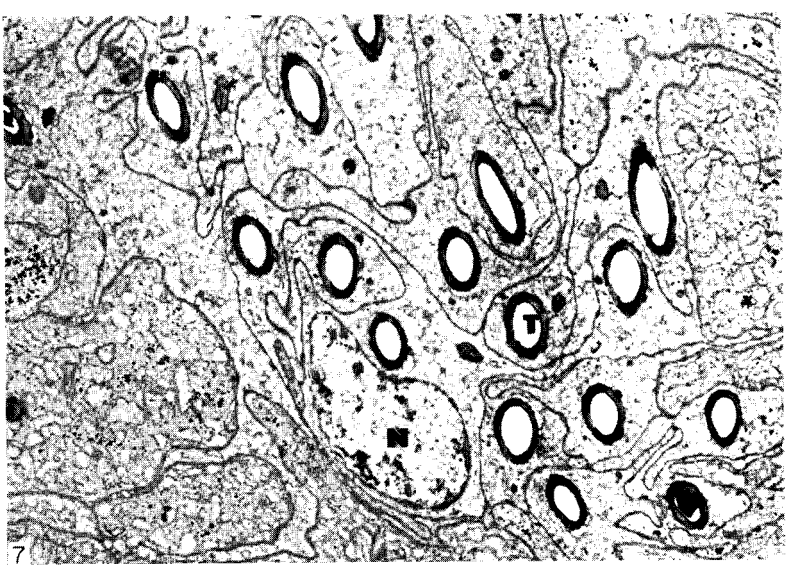

FIG. 7. EM section through a cluster of tubule-carrying cells. The nucleus (N) of one cell can be seen. Each chitinous tubule (T) lies within its own cell.

organelles such as Golgi bodies or ribosomal structures are only infrequently encountered.

Type 2b: The duct from cell 2a passes into the adjacent cell type 2b where it expands into a second cuticular organelle with many diverticula (Fig. 6). This central organelle is surrounded by many filaments (F) of a consistent and characteristic appearance which lie in a granular matrix that fills the central vesicle of cell 2b. Into this vacuole project the much shorter and less numerous microvilli of cell 2b. Many of these microvilli stain heavily at their distal ends. This cell also contains microtubules, especially near the cell membrane. In addition, the cytoplasm is often seen to contain dense granules reminiscent of glycogen granules.

A defined portion of the shared boundary between cell 2a and 2b seems to consist of septate junctions.

Tubule carrying cells: The cuticular organelle of cell 2b is continuous with the effluent duct which carries the secretion into the reservoir. Each of these ducts is the extracellular product of a tubule carrying cell(s) (Fig. 7), and their cuticle is continuous with the cuticle of the reservoir sac.

The secretory tissue of *Argoporis* seems to lack cell type 1 identified in *Eleodes longicollis*.

DISCUSSION

Though 6-methylnaphthoquinone, an anti-biotic agent, has been isolated from the fungus *Marasmius graminum* (BENDZ, 1951), 6-alkylnaphthoquinones have not been previously reported from the animal kingdom. Thus, among the alkyl benzoquinone-secreting tenebrionine beetles, the genus *Argoporis* stands out as unique in secreting alkyl naphthoquinones as well. In addition to *A. alutacea*, three other species of *Argoporis* (*A. costipennis* (Lec.), *A. bicolor* (Lec.), and *A. rufipes nitida* Casey) were examined. All secreted naphthoquinones in various ratios while other genera (*Eulabis*, *Apsena*, *Scaurus*, *Epantius*) of the tribe Scaurini (to which *Argoporis* belongs) did not do so, although each group produced compounds not found in other tenebrionids (Tschinkel, unpublished).

Morphologically, an obvious homology exists between the glands of *Argoporis* and those of other tenebrionine beetles. All are derived from the same intersegmental membranes, occupy similar positions in the body cavity, and show many similarities of arrangement of secretory tissues, ducts, valves, muscles, and other structures (LENGERKEN, 1925; ROTH, 1943, 1945; PALM, 1946; EISNER *et al.*, 1964; TSENG *et al.*, 1971; Tschinkel, in preparation). Ultrastructurally, while the general similarity and arrangement of the secretory cells of *Argoporis* and *Eleodes* justified the same nomenclature, the gland cells of *Argoporis* differ in some aspects from both *Eleodes* and *Zophobas* (TSCHINKEL, 1969). Thus, the highly vesiculated, almost 'frothy' appearance (which occurs consistently with a number of different fixatives) of the cytoplasm of *Argoporis* stands in contrast to the last two. Also, the microvilli completely fill the vesicular organelle of the 2a cell of *Argoporis* but do not even approach this condition in either *Eleodes* or *Zophobas*. The phenol-secreting cell of *Zophobas* does not appear to be associated with a second cell, so that it most closely

resembles the type 1 cells of *Eleodes*, a type apparently missing from *Argoporis*. Since the fine structures of so few tenebrionid defensive glands have been investigated, it is not possible to speculate on the correlation between ultrastructure and secretory products.

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REFERENCES

BENDZ G. (1951) An antibiotic agent from *Marasmius graminum*. *Ark. Kemi.* **3**, 495–500.

BUDZIKIEWICZ H., DJERASSI C., and WILLIAMS D. H. (1967) Quinones. In *Mass Spectrometry of Organic Compounds*, pp. 527–538. Holden-Day, San Francisco.

EISNER T., MCHENRY F., and SALPETER M. M. (1964) Defense mechanisms of arthropods—XV. Morphology of the quinone-producing glands of a tenebrionid beetle (*Eleodes longicollis* Lec.). *J. Morph.* **115**, 355–399.

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

KARNOVSKY M. J. (1965) A formaldehyde-glutaraldehyde fixative of high osmolality for use in electron microscopy. *J. Cell Biol.* **27**, 137A.

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

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* Duv.—II. The odoriferous glands of *Tribolium confusum*. *Ann. ent. Soc. Am.* **36**, 397–424.

ROTH L. M. (1945) The odoriferous glands in the Tenebrionidae. *Ann. ent. Soc. Am.* **38**, 77–87.

SCHILDKNECHT H., HOLOUBEK K., and KRAMER H. (1964) Defensive substances of arthropods, their isolation and identification. *Angew. Chem.* **3**, 73–82.

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. and WILLSON C. D. (1971) Inhibition of pupation due to crowding in some tenebrionid beetles. *J. exp. Zool.* **176**, 137–145.

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