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IRIDODIALS AND NEPETALACTONE IN THE DEFENSIVE SECRETION OF THE COCONUT STICK INSECTS, *Graeffea crouani*

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Abstract—The defensive secretion of the coconut stick insect, *Graeffea crouani* Le Guillou (Phasmatodea: Phasmatidae) from the Pacific Islands has, as major constituents: *trans,trans*- and *trans,cis*-iridodials and nepetalactone. *Cis,trans*-iridodial is a minor constituent. A minor iridoid has yet to be identified. Male and female insects yield the same constituents.

Key Words—Coconut stick insect, defensive secretion, *Graeffea crouani*, iridodial, nepetalactone, gas chromatography-mass spectrometry, Phasmatodea, Phasmatidae.

INTRODUCTION

Cyclopentanoid monoterpene derivatives have been reported from a wide range of insect and plant sources (Cavill, 1969, and references therein). Of these, the dialdehydes iridodial (I) and dolichodial (II) are well-known constituents of insect defensive secretions. Iridodial has been reported as a major component of the defensive secretion of various species of ants (cf. Cavill, 1969), and beetles (Vidari et al., 1973; Bellas et al., 1974; Fish and Pattenden, 1975). Dolichodial has also been isolated from several species of ants, while anisomorphal (dolichodial) was obtained from a phasmid or stick insect (Meinwald et al., 1962). More recently dolichodial was isolated

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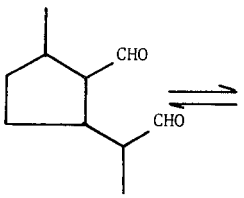
from a plant source, *Teucrium marum* (Pagnoni et al., 1976). The iridoid dialdehydes are structurally related to nepetalactone (III) (McElvain and Eisenbraun, 1955; Meinwald, 1954), the physiologically active principle of the catmint plant *Nepeta cataria* (Eisner, 1964), through the nepetalinic acids (IV, R = H), (Bates et al., 1958, and references therein).

The present paper reports on the chemistry of the defensive secretion of the coconut stick insect, *Graeffea crouani* Le Guillou (Phasmatodea: Phasmatidae), a widespread economic pest of coconut palms in the South Pacific region (Dumbleton, 1954; Swaine, 1971). The major constituents have been characterized as *trans,trans*-iridodial (VI),[4] *trans,cis*-iridodial (VII), and nepetalactone (III).



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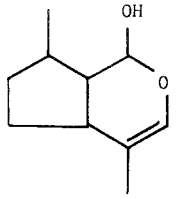

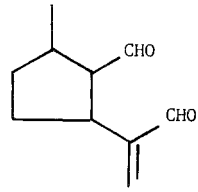

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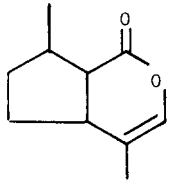

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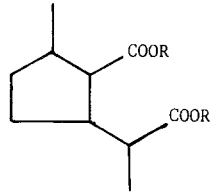

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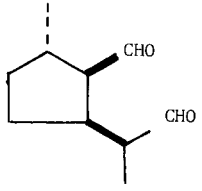

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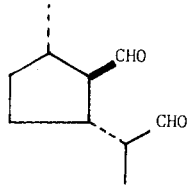

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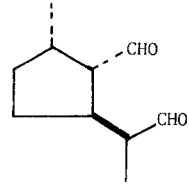

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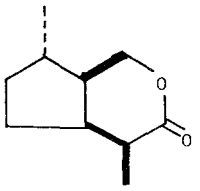
[4]In the designation of configuration, the relationship of the propional moiety to the formyl group is given first, and that of the formyl to the methyl group second, see V, VI, and VII.



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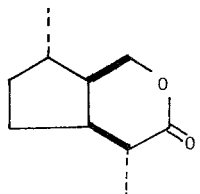

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METHODS AND MATERIALS

Isolation of Defensive Secretion of G. crouani. Samples of the secretion were collected by milking a colony of male and female stick insects, maintained on coconut fronds. The neck glands were squeezed to yield a milky secretion which was stored in sealed ampoules at −5°C; large females yielded up to 11 mg of secretion. When required, this neutral aqueous secretion was extracted with carbon tetrachloride.

Gas Chromatography and Mass Spectrometry. Analytical gas chromatography was carried out using a Perkin-Elmer F33 with a FID detector and fitted with glass columns (2 m × 3 mm) packed with: (1) 3% OV-101 on Gas Chrom Q, 80-100 mesh at 100° and 150°; and (2) 2.5% XE-60 on Chromosorb G, 80-100 mesh at 160°. Nitrogen was used as carrier gas (30 ml/min.).

Gas chromatography-mass spectrometry (GC-MS) (electron impact) was carried out using either a Shimadzu GC 6A, or a Varian 1740 gas chromatograph, with a flame ionization detector and helium as carrier gas. The GC was directly coupled to an AEI MS12 spectrometer using a straight-split separator. The MS was operated at 70 eV, ion source at 225°C. The following columns were used for GC-MS and for analytical purposes: (3) glass, 2 m × 3 mm, packed with 3% OV-1 on Gas Chrom Q, programed from 120° to 190° at 3° min^{-1}; (4) stainless steel, 2 m × 3 mm, 10% XE-60 on Chromosorb W at 145°; (5) glass 44 m × 0.5 mm Carbowax 20M, SCOT column at 160°; and (6) stainless steel, 2 m × 3 mm, 3% SE 30 on Chromosorb W at 140°.

Spectroscopy. Infrared and ultraviolet data were recorded on Perkin-Elmer 177 and Perkin-Elmer 402 instruments, respectively.

Reference Compounds. A mixture of *cis,trans-*, *trans, trans-*, and *trans,cis*-iridodials was synthesized by the method of Clark et al. (1959), starting from citronellal.

Derivatives. Oxidation of the total secretion was carried out using Jones' reagent, chromic acid in acetone (Bowden et al., 1946). The mixture of acids, when separated, was methylated with diazomethane.

RESULTS

The total secretion showed an absorption in the ultraviolet region, $\lambda_{max}(H_2O)$ 230 nm. The infrared spectrum showed $\nu_{max}(CCl_4)$ 2810, 2710, 1760, 1725, 1695, 1130 cm^{-1}, suggesting the presence of aldehyde and ester/lactone carbonyl groups.

On gas chromatography the secretion, and its extract in carbon tetrachloride, showed the presence of four volatile components (see Figure 1). Peaks 1, 2, and 3 represent major components, peak 4 was not observed in all samples. The retention times of the four peaks, on two columns, are consistent with these substances being monoterpenoids. There is no significant variation in the gas chromatographic data for the first three peaks between collections, or between samples of the secretion isolated from male and female phasmids.

After treatment with 2,4-dinitrophenylhydrazine, gas chromatography of the extract showed only peak 3 to be present. The carbonyl reagent has presumably formed derivatives with the components represented by peaks 1, 2, and 4. Purification by thin-layer chromatography and recrystallization yielded a yellow, crystalline 2,4-dinitrophenylhydrazone, mp 221–224°. The melting point was not depressed on admixture with a specimen of iridodial bis-2,4-dinitrophenylhydrazone, from *Iridomyrmex detectus* (Cavill et al., 1956).

Combined gas chromatography–mass spectrometry established that peaks 1 and 2 (Figure 1) are iridodials. Peak 1 shows M^+, m/e 168 (3%), and

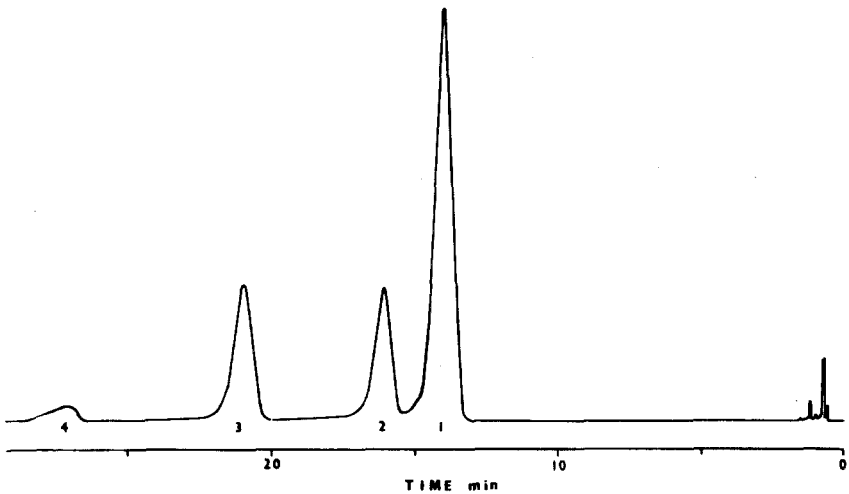

FIG. 1. Representative gas-liquid chromatogram of the fresh defensive secretion of *G. crouani*. Conditions column (1), isothermal 100°.

TABLE 1. LINEAR RETENTION INDICES OF IRIDODIALS AND DERIVED DIMETHYL NEPETALINATES

GC Column[a]	Iridodials of *G. crouani*	Reference iridodials: ex *I. nitidiceps*		Reference iridodials: Synthetic mixture		
		cis,trans-V	*trans,cis*-VII	*cis,trans*-V	*trans,trans*-VI[b]	*trans,cis*-VII
(3)	1246, 1271[c]	1235, 1247	1272	1238	1248	1272
(4) (Fig. 2)	1730, 1765, 1797[c]	1700, 1729	1757, 1804	1702, 1730	1766	1804

	Methylated oxidation Products of *G. crouani*	Reference dimethyl nepetalinates (IV, R = CH_3)		
		cis,trans-	*trans,trans*-[b]	*trans,cis*-[b]
(5)	1764, 1800, 1824	1759, 1781	1800	1818
(6)	1419, 1427, 1446	1412, 1430	1425	1442

[a]See Methods and Materials for description of column.
[b]Epimers in side chain not resolved.
[c]Nepetalactone (III) is also present LRI:1412 (3) and 1800 (4).

m/e 153 (3%), 150 (6), 135 (35), 111 (50), 109 (49), 95 (25), 93 (45), 81 (100), 71 (60), 67 (70), 58 (75), 55 (70), 43 (70), 41 (90). Peak 2 shows M^+, *m/e* 168 (2%), and *m/e* 153 (2%), 150 (6), 135 (25), 111 (60), 109 (45), 95 (25), 93 (35), 81 (100), 71 (70), 67 (70), 58 (60), 55 (50), 43 (70), 41 (90). These spectra closely correspond with that reported for iridodial, isolated from *Iridomyrmex nitidiceps* (Cavill et al., 1976).

Gas chromotographic comparisons and peak enhancement studies (see Table 1 and Figure 2) with authentic specimens of the *cis,trans*- and *trans,cis*-iridodials from *I. nitidiceps*, and with the synthetic *cis,trans*-V, *trans,trans*-VI and *trans,cis*-VII iridodials[5] show the presence of all three isomers in the defensive secretion of the coconut stick insect. Of these, the trans,trans isomer is the major constituent. The minor cis,trans isomer cochromatographs with *trans,trans*-iridodial on OV-101 (see Figure 1, peak 1).

Oxidation of the total secretion, using Jones' reagent, then methylation of the derived acids, gave a mixture of at least three methyl esters identified by GC-MS as dimethyl nepetalinates. Comparisons of linear retention indices with those for authentic specimens of the dimethyl nepetalinates (Cavill and McDonald, unpublished data) show the presence of the cis,trans-, trans,trans-

[5]Relative stereochemistry only is implied by these formulae. They are represented as corresponding in absolute configuration to the known iridoids of insect origin.

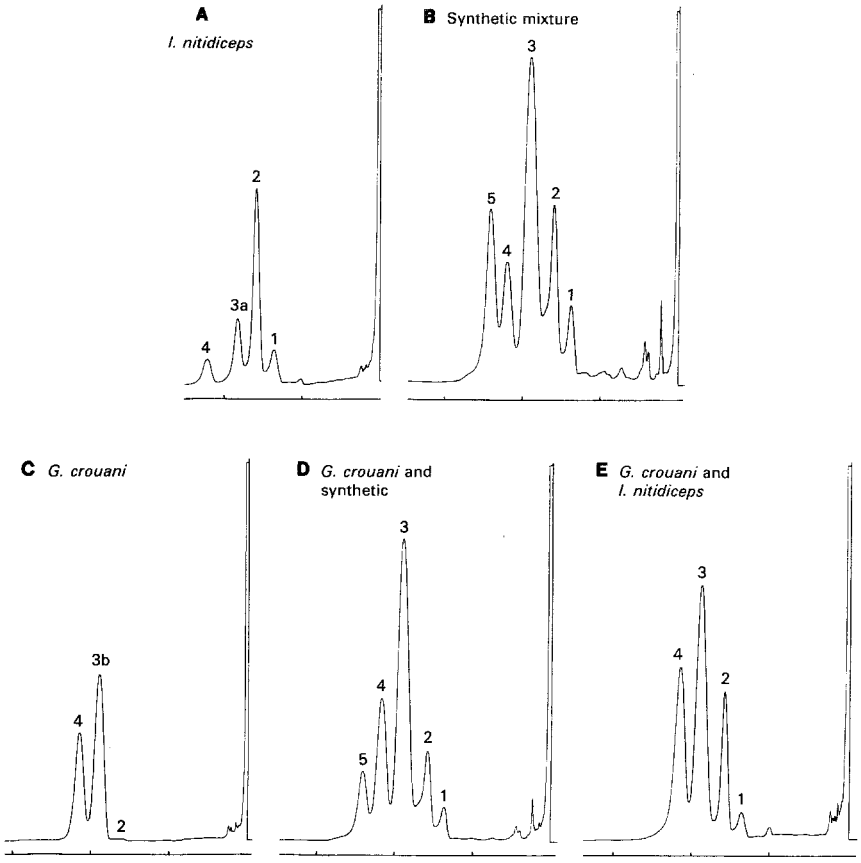

FIG. 2. Comparative gas-liquid chromatograms for the iridodials. Conditions column (4) isothermal 145°. Peak numbers correspond to the following iridodials: 1 and 2, epimers of cis,trans-; 3a and 4, epimers of trans,cis-; 3b, unresolved trans,trans epimers; 5, C_{10} acyclic dial. In 2C-E nepetalactone is also present in peak 4.

and trans,cis isomers (IV, R = CH_3) in the approximate ratio 1:30:10. Peak enhancement experiments confirm the presence of the two major isomers in the secretion from *G. crouani*. Methylation of the original secretion with diazomethane established that the acids were not present initially. Peaks 1 and 2 (Figure 1) are thus characterized as the *trans,trans-* plus *cis,trans-*, and the *trans,cis*-iridodials respectively.

Peak 3, on GC-MS, shows a strong molecular ion, M^+, *m/e* 166 (85%), and peaks at *m/e* 151 (10%), 138 (35), 137 (15), 123 (90), 121 (15), 110 (20), 109 (50), 107 (15), 95 (80), 81 (100), 69 (90), 67 (75), 55 (40), 53 (30), 43 (45), 41 (75). The spectrum differs from that reported for dolichodial (Cavill et al.,

1976), but closely corresponds to that of nepetalactone (Regnier, 1972). This assignment was confirmed by direct comparison of the above GC-MS data, and of linear retention indices on two columns, with that for an authentic specimen of nepetalactone from the catmint plant, *Nepeta cataria*. This nepetalactone was shown to contain the cis,trans and trans,cis-isomers (Bates and Sigel, 1963, and references therein).

The remaining compound, represented by peak 4 (Figure 1) is more polar. It did not react with diazomethane, nor was a derivative isolated on treatment with 2,4-dinitrophenylhydrazine. Peak 4, on GC-MS, does not show a molecular ion. The largest ion detected was at *m/e* 153 (35%), shown on high resolution to be $C_9H_{13}O_2$. Additional fragments were present at *m/e* 125 (13), 109 (5), 107 (5), 95 (13), 81 (30), 67 (12) and 43 (100%). This minor constituent would also appear to be an iridoid.

DISCUSSION

The defensive secretion of the coconut stick insect *G. crouani*, has as its major constituents the *trans,trans*-VI and *trans,cis*-VII iridodials and nepetalactone (III). The nepetalactone was shown to correspond, on gas chromatography, with naturally occuring nepetalactone from the catmint plant, *Nepeta cataria*, and hence is considered to be the cis,trans and/or trans,cis isomer (cf. Bates and Sigel, 1963). Only one species of a stick insect, *Anisomorpha buprestoides*, has been examined previously (Meinwald et al., 1962) from which anisomorphal (dolichodial) was characterized. Stereochemically anisomorphal has been shown to correspond to the trans,cis isomer of dolichodial (II) (Pagnoni et al., 1976).

Isolation of the known *trans,cis*-iridodial (VII), and a small proportion of the cis,trans-V isomer, from the defensive secretion of *G. crouani* is not unexpected in the light of the previous studies on *A. buprestoides*. The isolation of *trans,trans*-iridodial (VI), and nepetalactone (III), are reported for the first time from an insect source.

Recently *trans,trans*-dolichodial (type II) was characterized as a major constituent of the anal gland secretion of the Argentine ant, *Iridomyrmex humilis* (Cavill et al., 1976,) in this case in association with the known *cis,trans*-iridomyrmecin (VIII). The present isolation of three iridodials (V, VI, and VII) in association with nepetalactone (III) is consistent with the original biosynthetic scheme proposed for the iridoids (Clark et al., 1959; see also Cavill and Robertson, 1965). Nepetalactone may arise by enzymic oxidation of an enol-lactol tautomer of iridodial. An earlier attempt to achieve this oxidation using manganese dioxide in light petroleum was unsuccessful (Cavill and Ford, 1960).

Iridodial isomer variation has been reported for three species of

American dolichoderine ants (McGurk et al., 1968). Each of the three species studied contained more than 80% of a single cis,trans or trans,cis isomer, the remaining cis,trans- and trans,cis isomers being minor constituents. Two of the species also produced an iridolactone-iridomyrmecin (VIII) and isoiridomyrmecin (IX), respectively, of the same configuration as the major iridodial isomer. The biological significance, if any, of such iridodial isomer variation and of the present data for the predominant *trans,trans*- and *trans,cis*-iridodials in the coconut stick insect is not known.

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