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CHRYSOMELIDIAL IN THE DEFENSIVE SECRETION OF THE LEAF BEETLE *Gastrophysa cyanea* MELSHEIMER

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Abstract—Larvae of the chrysomelid beetle *Gastrophysa cyanea* produce a defensive secretion in their eversible thoracic and abdominal glands that is an effective repellent for small predators such as fire ants. This secretion is composed primarily of chrysomelidial, 2-(2-formyl-3-methyl-2-cyclopentenyl)propanal, and a compound tentatively identified as its enol lactone. Adaptations that optimize the effectiveness of the larval defensive exudate are discussed.

Key Words—chrysomelidae, chrysomelidial, *Gastrophysa cyanea*, defensive secretion.

INTRODUCTION

The eversible glands of larval leaf beetles (Chrysomelidae) have proven to be a rich source of insect defensive products. A number of diverse compounds, including salicylaldehyde (Wain, 1943, Pavan, 1953; Wallace and Blum, 1969), benzaldehyde (Moore, 1967), and β-phenylethyl esters (Blum et al., 1972), have been identified in the secretions of the relatively few species of chrysomelid larvae that have been so far investigated. We have investigated the defensive secretion of *Gastrophysa cyanea* and have identified as its major constituent chrysomelidial, a novel cyclopentenoid monoterpene aldehyde.

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METHODS AND MATERIALS

The secretion was collected in 0.5-μl microcapillaries from the everted glands of tactually stimulated larvae, and stored in *n*-hexane or methylene chloride at $-10°$. These extracts were used directly for all chemical analyses.

The secretion was analyzed on the following gas-liquid chromatography columns: 10% Carbowax 20 M, 3% SP-1000, and 3% ECNSS-M. Combined gas chromatographic–mass spectrometric (GC–MS) analyses were carried out on an LKB 9000 instrument. Nuclear magnetic spectra were obtained on a Varian XL-100-15 spectrometer equipped with a Digilab Fourier transform system.

The deterrent value of the defensive secretion was examined by placing larvae on the foraging platform of a fire ant (*Solenopsis invicta*) colony and observing the subsequent confrontations. The repellency of chrysomelidial, collected by preparative gas chromatography, was examined by treating *Tenebrio* larvae with 10 μg of this compound and noting the reactions of fire ant workers that encountered these treated larvae placed on their foraging platform.

RESULTS

Identification of Chrysomelidial

Upon GC–MS, the extract showed two peaks of nearly equal height eluting at 10.6 and 15.5. min at 160° on a 2-m 10% Carbowax 20 M column. The first peak gave a molecular ion at *m/e* 164 (65), and important ions at *m/e* 136 (60), 121 (40), 107 (100), 93 (45), 91 (76), and 80 (82), whereas the second peak gave a molecular ion at *m/e* 166 (6) and important ions at *m/e* 151 (4), 148 (21), 138 (15), 137 (4), 133 (6), 123 (10), 109 (53), 108 (26), 81 (100), and 79 (37).

After isobutane chemical ionization, mass measurement of the two protonated molecular ions led to the formulas $C_{10}H_{12}O_2.H^+$ (found 165. 0925) and $C_{10}H_{14}O_2.H^+$ (found 167.1093), respectively. Neither compound reacted with silanizing agents, but the peak from the second compound disappeared after treatment with $NaBH_4$. Furthermore, the second compound reacts with 2,4-dinitrophenylhydrazine and Purpald (Dickinson and Jacobson 1970) (after collection from the gas chromatograph) and forms a dimethoxime exhibiting a molecular ion at *m/e* 224 (4) and other ions at *m/e* 193 (7), 178 (9), 138 (100), 107 (38), 106 (40), 87 (15), and 79 (23). The base peak at *m/e* 138 (M-86) and the rearrangement ion at *m/e* 87 in the mass spectrum of the methoxime, taken with the intense peak at *m/e* 109 (M-57, confirmed by a

metastable ion at 71.7) in the original compound, point to the structural feature $-CHCH_3CHO$ and indicate the presence of a γ-hydrogen atom.

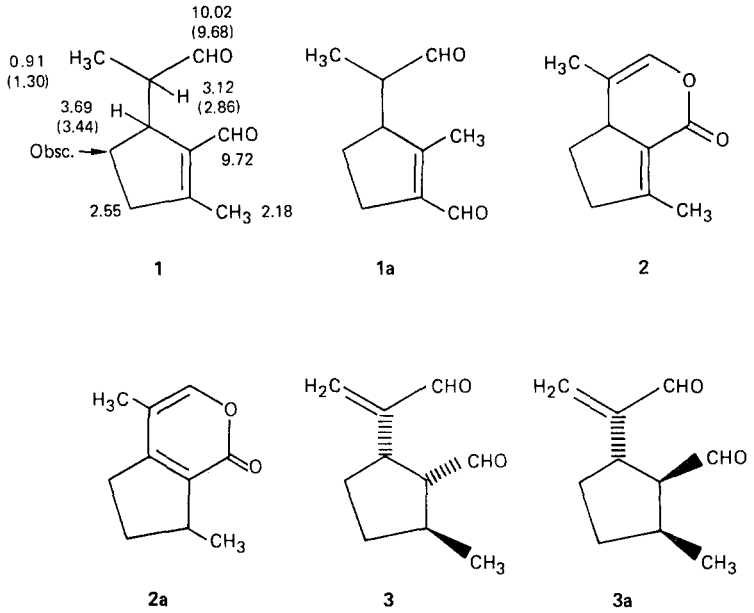
Preparative gas chromatography of this second peak provided enough material (~50 μg) for a Fourier-transform PMR (100 mHz, $CDCl_3$, 35,000 16K free induction decays). Two aldehydic protons at δ9.72 and 10.02, were clearly visible along with an olefin-bound methyl (δ2.18) weakly coupled to allylic protons ($\nu_{1/2}$ 6 Hz). In confirmation of the mass spectral results, a doublet was observed at δ0.91 (J~7 Hz) for the methyl protons of the $-CHCH_3CHO$ feature. The corresponding α proton (δ3.12) was additionally coupled to a downfield, presumably allylic hydrogen at δ3.69 (J~4 Hz). Finally, an allylic methylene triplet was observed at δ2.55, further broadened by long-range coupling. The remaining methylene group probably appeared in the δ1.5–1.7 region, but was obscured by impurities in the solvent. The lack of olefinic protons observed indicates a ring.

The presence of a tetrasubstituted, α,β-unsaturated aldehyde was suggested by the ultraviolet spectrum (λ_{max}^{Etoh} 256 nm). This absorption disappeared on treatment with $NaBH_4$ and shifted to 277 nm (285 nm infl.) on treatment with semicarbazide. There appear to be few models for a 2-substituted 1-cyclopentene carbaldehyde,[4] but the values for 2-methyl-1-cyclohexene carbaldehyde (λ_{max}^{Etoh} 242 nm, ε 11,000) (Braude and Timmons, 1955), 1-cyclohexene carbaldehyde (λ_{max}^{Etoh} 229 nm, ε 12,000) (Heilbron et al., 1949), and 1-cyclopentene carbaldehyde (λ_{max}^{Etoh} 237 nm, ε 12,000, semicarbazone 267 nm, ε 37,500, 277 nm infl., ε 25,500) (Heilbron et al., 1949) suggest that a value near 250 nm is reasonable.

These facts can be accommodated by structures 1 or 1a for chrysomelidial; the existence of the closely related dolichodial (3 and 3a) (Cavill and Hinterberger, 1961; Cavill and Whitfield, 1962; 1964; Cavill, 1969) and anisomorphal (3 or 3a) (Meinwald et al., 1962) leads us to favor the former, and we have assigned the resonances accordingly.[5] The lack of observable coupling in structure 1 between the aliphatic aldehyde and its adjacent H indicates that the methyl and carbonyl are essentially eclipsed, as expected for an α-substituted aldehyde (Karabatsos and Hsi, 1965). The downfield shift of the adjacent allylic hydrogen is unusual, and models suggest that it may be caused by the influence of the nearby unsaturated aldehyde. Its mass

[4] Carbaldehyde replaces carboxaldehyde according to IUPAC, nomenclature rules; c.f. J.H. Fletcher, O.C. Dermer, and R.B. Fox, *Nomenclature of organic compounds*, Advances in Chemistry Series #126, American Chemical Society, Washington, D.C., 1974, p. 161.

[5] After this work was completed, we learned that J. Meinwald had identified chrysomelidial in the secretion of another chrysomelid larva, a *Plagiodera* species. He has kindly compared a sample of chrysomelidial isolated from *G. cyanea* to an unambiguously synthesized sample of this compound and found them to be identical; cf. J. Meinwald, T.H. Jones, T. Eisner, and K. Hicks, *Proc. Natl. Acad. Sci.* 74:2189–2193 (1977).

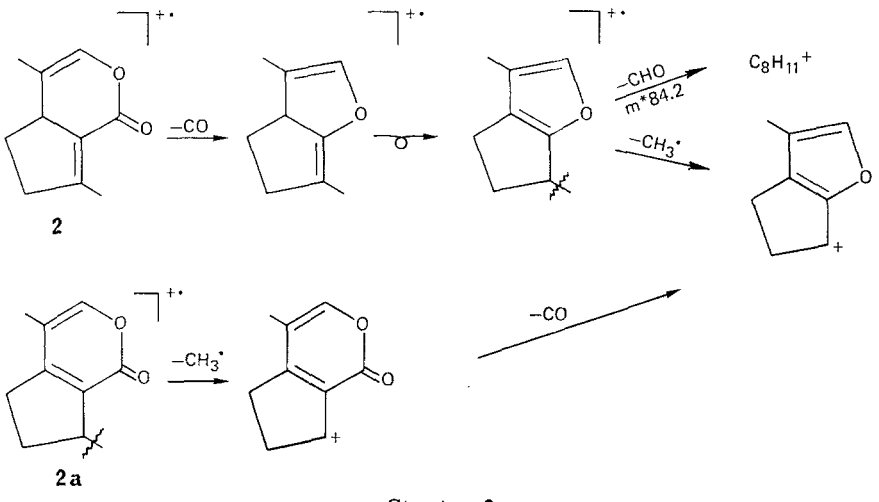


Structure 1

spectrum, involving loss of the sidechain, is, of course, markedly different from that of dolichodial. By analogy with dolichodial (Cavill and Hinterberger, 1961), hydrogenation of this compound (PtO_2–ethanol) might be expected to produce the known dihydro derivative, iridodial, or one of its isomers. Unfortunately, however, extensive hydrogenolysis occurs, giving at least seven compounds, none of which show a mass spectrum similar to that of irododial.

A second sample of chrysomelidial collected from a 3% SP-1000 GC liquid phase showed the presence of a second, very similar compound via a doubling of several major PMR peaks. When the sample was reexamined with a 3% ECNSS-M column, two GC peaks of similar intensity appeared at 14.2 and 15 min, giving nearly identical mass spectra. It is clear that the carbon alpha to the aliphatic aldehyde epimerized during GC in the presence of the somewhat acidic SP-1000 liquid phase. We have assigned to this epimer the NMR peaks shown in parentheses on structure 1.

With the later-eluting peak of the extract established as resulting from structure 1, the first peak, whose mass spectrum lacks two hydrogens compared with 1, may represent the corresponding enol lactone, 2. Its mass spectrum, characterized by loss of CO followed by loss of either methyl or CHO, may be explained as follows:



Structure 2

An old sample of the extract exhibited a trace of an isomer of 2 eluting just before 1 on 3% ECNSS-M and having an intense molecular ion at *m/e* 164 (100) and important ions at *m/e* 149 (64), 135 (45), 121 (53); i.e., loss of the same fragments occurred in the reverse order. We suggest this is the conjugated pyrone 2a, which would not require rearrangement prior to methyl loss as would 2.

Defensive Value of the G. cyanea *Secretion*

Mature larvae of *G. cyanea* that had been placed on the foraging platform of a fire ant colony were generally avoided by these normally aggressive ants. However, the occasional ant workers that attacked the larval beetles were totally disarmed by the chrysomelid secretion. Larvae that were tactually stimulated by their ant antagonists immediately evaginated their eversible glands, smearing their aggressors with the defensive secretion. Typically, a contaminated ant worker immediately withdrew from the scene of the encounter, dragging its head and antennae, and moving in a completely disoriented manner. The secretion-labeled ant was avoided by its sister workers. Furthermore, when a secretion-moistened larva fresh from an encounter moved near a group of ant workers feeding on a cockroach, the ants immediately abandoned their repast and moved rapidly from the area.

Tenebrio larvae treated with chrysomelidial were avoided by ant workers for 10 min or longer. On the other hand, untreated larvae were immediately overrun by ant workers.

DISCUSSION

The identification of chrysomelidial in the glandular exudate of *G. cyanea* further emphasizes the diversity of defensive compounds produced by larval Chrysomelinae. Salicylaldehyde dominates the secretions of species of *Phyllodecta* (Wain, 1943), *Melasoma* (Pavan, 1953), and some species of *Chrysomela* (Wallace and Blum, 1969). On the other hand, the defensive secretion of *Chrysomela interrupta* is comprised primarily of β-phenylethyl isobutyrate and β-phenylethyl 2-methylbutyrate (Blum et al., 1972). It would not prove surprising if the defensive exudates of species in other chrysomelid genera proved to be sources of interesting new insect natural products.

We consider the secretion of *G. cyanea* to be the most potent fire ant deterrent produced by any species of chrysomelid that we have examined. Several behavioral adaptations appear to optimize the effectiveness of the chrysomelidial-rich defensive exudate of *G. cyanea*. Early-instar larvae characteristically aggregate on the undersides of dock (*Rumex* sp.) leaves, where they are very inconspicuous. In a sense, the clumped larvae "pool" their limited defensive secretion. Disturbance of a larval aggregation results in the virtual simultaneous eversion of the glands of many larvae. In young larvae, only the thoracic glands are functional, whereas in older larvae, which are often solitary, the defensive secretion issues from glands through pairs of tubercles on the last two thoracic and first seven abdominal segments as has been reported for other species of Chrysomelinae (Garb, 1915).

Significantly, the pupae of *Gastrophysa*, unlike those of *Chrysomela* species, shed their pupal skin entirely, thus losing their defensive glands. *Chrysomela* pupae, which are exposed on the host plant, retain the larval integument that contains the salicylaldehyde-rich defensive glands. The glands can be discharged when the freehanging pupa is stimulated (Hinton, 1951; Wallace and Blum, 1969). Indeed, the freshly emerged adult, which is especially vulnerable to predators, is bathed in the salicylaldehyde derived from the retained larval defensive glands, and this aromatic bath renders the beetle highly repellent to invertebrate predators at a time when it is very inactive (Wallace and Blum, 1969). *G. cyanea*, on the other hand, discards its chrysomelidial-fortified glands when it pupates, and would seem to be especially susceptible to predation if exposed like the pupa of *Chrysomela*. However, the *Gastrophysa* larvae pupate in the ground litter near the host plant, and are very unobtrusive. These results illustrate the variety of defensive mechanisms evolved in the Chrysomelidae and emphasize the chemical and behavioral diversity manifested by the species in this large family.

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