Tetrahedron Letters No. 1, pp. 29-33, 1962. Pergamon Press Ltd. Printed in Great Britain.

DEFENSE MECHANISMS OF ARTHROPODS - IX

ANISOMORPHAL, THE SECRETION OF A PHASMID INSECT[1]

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(Received 9 December 1961)

THE southern walking-stick, Anisomorpha buprestoides (Stoll), is a large nocturnal herbivorous insect, long known for its habit of ejecting a defensive spray when it is handled or otherwise disturbed. The secretion is strongly irritating to the exposed surfaces of the face, and has in fact been reported to cause transient but serious injury to the eyes.[2] The present paper deals with the chemistry of the active principle of the spray, hereafter called anisomorphal, which has hitherto never been investigated. The more purely biological aspects of the problem, *viz*. the anatomy and mechanics of operation of the glandular apparatus, the ability of the insects to aim their spray accurately toward the source of disturbance, and the effectiveness of the secretion as a means of defense against predators, will all be considered in a forthcoming paper of this same series.

The secretion was collected in pure form by causing individual walking-sticks to spray directly into test tubes and then immediately rinsing out the secretion with methylene chloride. Over one thousand milkings were made in this way, from a total of several hundred walking-sticks. Although the glands are present in both sexes and in all nymphal stages, adult females

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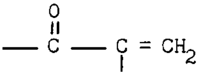
[1] The support of this research by grants E-2908 and E-1210 from the National Institutes of Health is acknowledged with pleasure.

[2] M.A. Stewart, *Canad. Entomol.* **49**, 84 (1937).

were used exclusively, simply because of their larger size and correspondingly greater secretory output. Some of the milking was done in the field when the animals were first collected,[3] but most of it was carried out later with a laboratory colony maintained on privet (Ligustrum sp.). Although in nature the walking-sticks appear to feed primarily on a variety of oaks, the shift to a different diet in captivity proved to have no noticeable effect on the chemistry of the secretion.

After drying the methylene chloride solution (the secretion contains a substantial amount of water), and evaporating the solvent under reduced pressure, the residue appeared on the basis of gas chromatographic evidence to be essentially a single component. Its infrared spectrum showed characteristic maxima corresponding to aldehydic C-H (3.71μ), an isolated carbonyl function (5.82μ), and a second carbonyl function (5.93μ) conjugated with a double bond (6.20μ).[4] The ultraviolet absorption spectrum (λ_{max}^{EtOH} 223 mμ, ε 7.910) confirmed the presence of a conjugated enone chromophore, and furthermore indicated that this chromophore must have a low degree of substitution.[5]

In the near infrared, a well-defined band at 1.627μ corresponding to a terminal methylene group[6] suggested that this chromophore corresponds to the moiety shown in part-structure I.



$$-\overset{O}{\overset{\|}{C}}-\underset{|}{C}=CH_2$$

I

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[3] The walking-sticks were collected in Florida on the grounds of the Archbold Biological Station, Highland Co., and the University of Florida Conservation Reserve, Welaka. Thanks are due the Directors of these Institutes, Messrs. Richard Archbold and William M. Dunson, for making their facilities available to us.

[4] L.J. Bellamy, The Infra-red Spectra of Complex Molecules. Methuen, London (1958).

[5] A.E. Gillam and E.S. Stern, Electronic Absorption Spectroscopy. Arnold, London (1954).

[6] R.F. Goddu in C.N. Reilley's Advance in Analytical Chemistry and Instrumentation Vol. 1, pp. 347-424. Interscience, New York (1960).

The NMR spectrum of this secretion revealed a pair of peaks of equal intensity at 4.03 and 3.70 τ in good agreement with expectations for the β-olefinic protons of I. Additional peaks at 0.45 and 0.23 τ (doublet) showed that both carbonyl functions must be aldehydic.[7]

The small amount of secretion available, combined with its tendency to decompose during chromatography or micro-distillation, precluded a direct elementary analysis. However, the formation of a crystalline 2,4-dinitrophenylhydrazone, whose elementary analysis corresponded to that of a bis-derivative of a molecule with the composition $C_{10}H_{14}O_2$, provided chemical evidence that the defensive secretion was a terpene dialdehyde. In view of the instability of the secretion itself, it was considered useful to reduce it to a more stable derivative. Reduction of the dialdehyde with sodium borohydride gave the corresponding diol, which was partially reoxidized by manganese dioxide[8] to a monoaldehyde monoalcohol,[9] thus providing additional support for the arrangement of functional groups deduced earlier. Catalytic reduction of the diol resulted in hydrogenolysis of the allylic alcohol function, as well as hydrogenation, giving a saturated alcohol. This product had a characteristic odor strongly reminiscent of 1-hydroxymethyl-2-isopropyl-5-methylcyclopentane (II), which by a fortunate coincidence was first characterized in these laboratories as a degradation product of the active constituent of catnip, nepetalactone (III).[10] The infrared spectra of two samples, obtained by degradation of the insect product and

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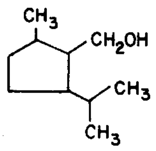
[7] L.M. Jackman, Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry. Pergamon Press, London, New York (1959).

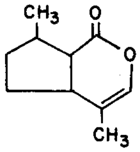
[8] R.M. Evans, Quart. Rev. 13, 61 (1959).

[9] The course of this oxidation was unusually complex, since a small amount of saturated carbonyl (5.82 μ) appeared in the infrared spectrum of the product, along with the expected hydroxyl (2.80, 2.90 μ) and α,β-unsaturated carbonyl (5.93, 6.20 μ) absorption bands.

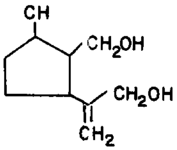
[10] J. Meinwald, Chem. & Ind. 488 (1954); J. Meinwald, J. Amer. Chem. Soc. 76, 4571 (1954).

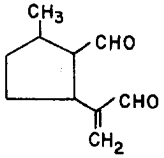
of the plant oil, were very nearly superimposable. Furthermore, retention times on two different gas chromatographic columns were identical. On this

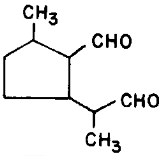

II


III

basis, it was tentatively concluded that our reductive degradation product had structure II. Once this point is accepted, it follows that the allylic diol must be IV, and the dial itself V.


IV


V


VI

While our work was in progress, Cavill and Hinterberger described the isolation of a new terpene, dolichodial, from the crushed bodies of Australian ants (species of Dolichoderus and Iridomyrmex).[11] On the basis of entirely independent evidence, these authors were able to assign structure V to dolichodial. The relationship between anisomorphal and dolichodial is unclear. The difference in specific rotations ($[\alpha]_D$ +3.8° for anisomorphal vs. $[\alpha]_D$ -26° for dolichodial) might indicate (1) that the two substances are diastereoisomers or (2) that they are enantiomorphic but in the case of anisomorphal partially racemic or (3) that they are really identical but at least in one case contaminated with an optically active impurity. Although

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[11] C.W.K. Cavill and H. Hinterberger, XI Internationaler Kongress für Entomologie Verh. B. III, 285 (1960); C.W.K. Cavill and H. Hinterberger, Austr. J. Chem. **14**, 143 (1961).

anisomorphal appears homogeneous when subjected to gas chromatography or thin layer chromatography, we have not had sufficient amounts to attempt rigorous purification. Other than simple distillation, no evidence for the homogeneity of dolichodial has been presented.

Despite the stereochemical ambiguity, the structure of anisomorphal was confirmed by the close correspondence of its infrared and ultraviolet spectra with those reported for dolichodial, as well as by the melting point and mixture melting point behavior of the 2,4-dinitrophenylhydrazones.[12] Finally, hydrogenation of anisomorphal gave iridodial (VI), characterized as its 2,4-dinitrophenylhydrazone by comparison with an authentic sample.

The occurrence of two closely related terpenes, dolichodial and anisomorphal in ants and phasmids - two groups of insects widely separated phylogenetically from one another - may seem surprising and might be taken to suggest that the ability to synthesize these molecules has evolved independently in each group. However, not enough is known about terpene production in insects to rule out the possibility that the underlying biosynthetic machinery is actually widespread among the class Insecta.

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[12] We wish to thank Dr. Cavill for supplying us with authentic samples of the 2,4-dinitrophenylhydrazones of dolichodial and iridodial.