phases. However, if the volumes were "normal," for example if ΔV is constant, the situation would improve greatly, as the line $\Delta V = 0.514$ cm^3 shows in Fig. 3. For larger ΔV's in different transitions this type of absolute volume error would play a less significant role. It is only in cases such as this, with a comparatively small ΔV at 1 atm, that anomalous volume behavior at high pressure may lead to gross inaccuracies in phase predictions.

JOHN C. JAMIESON
BART OLINGER

Department of Geophysical Sciences, University of Chicago, Chicago, Illinois 60637

References and Notes

1. R. G. McQueen, J. C. Jamieson, S. P. Marsh, *Science* **155**, 1401 (1967).
2. A. Navrotsky, J. C. Jamieson, O. J. Kleppa, *ibid.* **158**, 388 (1967).
3. N. A. Bendeliany, S. V. Popova, L. F. Vereshchagin, *Geokhimiya* **5**, 499 (1966); P. Y. Simons and F. Dachille, in *Program, Annual Meeting of Geol. Soc. Amer.* (1966), abstr., p. 203.
4. L. F. Vereshchagin, S. S. Kabalkina, A. A. Kotilevets, *Soviet Phys. JETP* (*English Transl.*) **22**, 1181 (1966); S. S. Kabalkina, L. F. Vereshchagin, L. M. Lityagina, *Soviet Phys. Doklady* (*English Transl.*) **12**, 946 (1968).
5. J. C. Jamieson, in *Physics of Solids at High Pressure*, C. T. Tomizuka and R. M. Emrick, Eds. (Academic Press, New York, 1965), p. 444.
6. D. L. Decker, *J. Appl. Phys.* **37**, 5012 (1965).
7. H. E. Swanson and E. Tatge, *U.S. Nat. Bur. Stand. Circ. No. 539* (1953), vol. 1.
8. S. P. Clark, Ed., *Handbook of Physical Constants* (Geological Society of America, rev. ed., Mem. 97, New York, 1966).
9. D. T. Cromer and K. Herrington, *J. Amer. Chem. Soc.* **77**, 4708 (1955).
10. We wish to thank Dr. O. J. Kleppa for his valuable criticism.
11. Supported by NSF grant GP 3351, grant SD-89-Research from Advanced Research Projects Agency, and the Petroleum Research Fund of the American Chemical Society grant 12 PRF-1408-06. Grateful acknowledgment is made to the donors of that fund.

8 April 1968

Juvenile Hormone: Activity of Natural and Synthetic Synergists

Abstract. *A group of nonsesquiterpenoid compounds currently used commercially as insecticide synergists possesses a high order of juvenile hormone activity and species specificity.*

Certain compounds not in themselves appreciably toxic to insects increase the toxicity of such insecticides as pyrethrins and carbamates. Such compounds are commonly referred to as adjuvants or synergists.

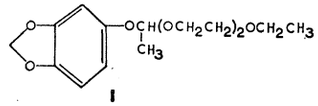
Recent interest in the use of insect hormones, particularly juvenile hormones, as insect control agents prompted me to examine the effects of several well-known synergists with 10, 11-epoxyfarnesenic acid methyl ester (*1*), an analog of the cecropia juvenile hormones, to see whether its activity could be augmented.

In preliminary tests, juvenile hormone activity was increased somewhat by the combination of hormone and synergist, but control insects treated with the synergist alone also manifested the unmistakable morphogenetic effects associated with juvenile hormone activity. The biological activity of several synergists and related analogs have now been examined.

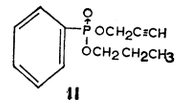
The activities of all compounds were determined in *Tenebrio* and milkweed bug assays (*2*, *3*). Juvenile hormone activity is measured by treating the penultimate stage of the insect with the test compound and examining it after its last molt toward the adult form for the retention of immature features. Freshly molted pupae of the yellow mealworm, *Tenebrio molitor* (L.), and last instar nymphs of the milkweed bug *Oncopeltus fasciatus* (Dallas), are treated topically on the venter of the abdomen with the test compounds in 1 μl of acetone and held until the following molt when juvenile hormone activity is signaled by the presence of immature characters (Figs. 1 and 2).

Initial studies were made with technical products; but, any which were active at 100 μg were purified by column chromatography to a purity greater than 99 percent so that specific activity could be determined more accurately. Purity was ascertained by a combination of thin-layer and gas-liquid chromatography. Sesamin and sesamolin were isolated from sesame oil by chromatography over Florisil (*4*) and recrystallization; they were judged pure on the basis of melting point, ultraviolet and infrared spectra, and gas-liquid chromatography. Because sesamin and sesamolin are insoluble in acetone, they were tested by application in 0.25 μl of Carbowax 200.

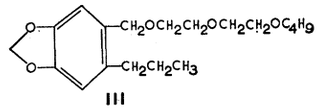
The specific activity of representative compounds is presented in Table 1. In the *Tenebrio* test, the most active synergist was sesoxane (I) which produced second pupae at each dose. Subsequent tests at 0.75, 0.5, and 0.25 μg gave 4, 4, and 3 degrees of activity, respectively. The Niagara synergist, propyl 2-propynyl phenyl phosphonate (Niagara 16388) (II), was nearly as active as sesoxane and somewhat more active than piperonyl butoxide (III). Of the remaining synthetic synergists, MGK 264 [*N*-(2-ethylhexyl)-5-norbornene-2,3-dicarboximide] and bucarpolate [2-(2-butoxyethoxy) ethyl piperonylate] caused only nominal activity. In contrast, the naturally occurring synergists sesamin (IV) and sesamolin (V) were nearly inactive (sesamin had slight activity at 100 μg).



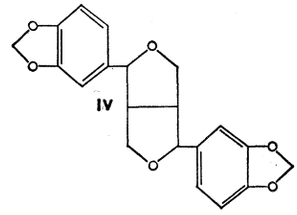
I

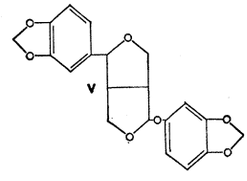


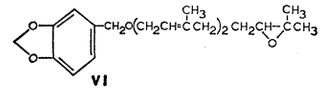
II



III


IV


V



VI

The milkweed bug assays provided interesting information on species specificity reminiscent of results obtained with the monocylic sesquiterpenoid ester juvabione (*3*, *5*). Metamorphosis was inhibited in the milkweed bug by each of the compounds active on *Tenebrio* except NIA-16388, and by several compounds which were ineffective on *Tenebrio*, that is, MGK 264, tropital, piperonal bis [2-(2-butoxyethoxy)ethyl] acetal, and sesamolin. With the exception of MGK 264 and NIA-16388, all of the compounds active on *Tenebrio* and the milkweed bug were derivatives of methylenedioxyphenyl (MDP) which differ principally with respect to the side chain substituents. Derivatives of MDP lacking a side chain (safrole, piperonyl alcohol, piperonal) were ineffective. Derivatives of MDP without

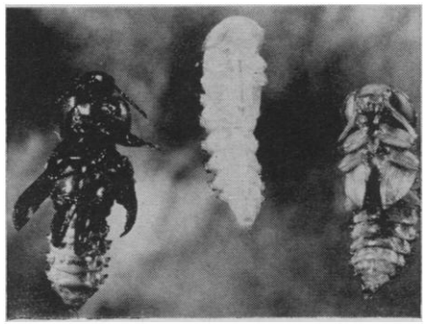


Fig. 1. Juvenilization of mealworm pupae. A normal pupa (center) responded to treatment with a juvenilizing chemical by molting to a pupal adult intermediate (left) or underwent a second pupal molt to form a second pupa (right).

a side chain are also ineffective for insecticide synergism (*6*).

In light of the apparent importance of the side chain to activity and species specificity, MDP derivatives of two sesquiterpenoid structures of known juvenile hormone activity were synthesized. Gentle refluxing of piperonyl alcohol in anhydrous dimethoxyethane with potassium tert-butoxide and farnesyl chloride gave piperonyl farnesyl ether in good yield. The epoxide (VI) was prepared by treatment with *m*-chloro perbenzoic acid (*1*). Purification of these compounds was accomplished by chromatography over Florisil, and the structures of each were confirmed by infrared, ultraviolet, nuclear magnetic resonance, and mass spectra data.

Both species of test insects responded to these compounds, particularly the milkweed bug. The increase in activity after formation of the epoxide at the 10,11 position in the farnesol moiety in both species was a predictable result since such a process increases the activity of farnesol, farnesyl methyl ether, methyl farnesenate (*1*), and a C_{17} terpenoid ester isolated from the adult male cecropia moth, *Hyalophora cecropia* (L.) (*7*).

Also, ethers of several compounds (that is, dodecanyl methyl ether and farnesyl methyl ether) possess a high order of juvenile hormone activity (*2*, *8*) only when injected. In my study, the topically applied MDP ethers were very active.

As noted, two of the active synergists (NIA-16388, MGK 264) are not MDP derivatives. Therefore the exact contribution of the MDP group to activity is unclear; nevertheless several structurally dissimilar synergists and the MDP-farnesyl ether derivatives possess juvenile hormone activity identical to that of previously known nonaromatic sesquiterpenoid derivatives, which argues for a common site of activity within the insect.

Although the juvenile hormone activity of the synergists duplicated in all respects the morphogenetic effects of the known active sesquiterpenoid derivatives, it seemed possible that the synergists might be producing their effects by activating or turning on the insect's own corpora allata and stimulating the production of its own juvenile hormone. To examine this hypothesis, I severed ten 1-day-old *Tenebrio* pupae at the thoracic-abdominal juncture, sealed the wounds with a mixture of melted paraffin and petroleum jelly, and treated each isolated fragment topically with 2 μg of sesoxane in 1 μl of acetone. Controls were treated with acetone only. If the insect's own corpora allata were activated by the synergist, only the head and thorax would be expected to molt and show juvenilization since the abdominal fragment contains no known endocrine glands. After approximately 7 days, all of the fragments treated with sesoxane had molted, completely retaining pupal characters, indicating clearly that the activity of the synergist was directly related to the compound itself and not to activation of the insect's corpora allata. Control fragments molted and attained normal adult morphology.

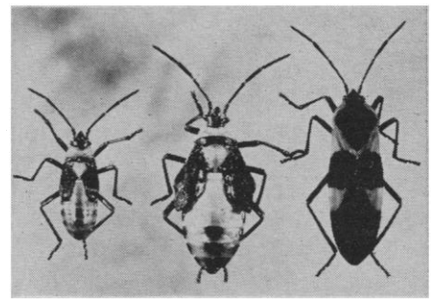


Fig. 2. Juvenilization of milkweed bug. Treatment of a last instar nymph (left) with a juvenilizing compound resulted in the formation of a supernumerary giant nymph (center) at the next molt instead of a normal adult (right).

The synergists piperonyl butoxide and sesoxane increase the toxicity of pyrethrins and carbamate insecticides by inhibiting microsomal oxidation and hydroxylation enzymes which ordinarily account for the rapid metabolism and inactivation of these insecticides (*9*); the juvenile hormones may exert their effects by regulating these or similar enzymes. Perhaps related to this, I have found that 4 to 8 μg of a synthetic analog of ecdysone, Δ^7-5β-cholestene-2β,3β,14α-triol-6-one (prepared in our laboratory by M. J. Thompson), injected into *Tenebrio* pupae produced pupal-adult intermediates. This compound is similar to α-ecdysone except that hydroxyl groups at the 22 and 25 positions are lacking. In light of this information, it does not seem unduly speculative to suggest that the juvenile hormones and the synergists may inhibit hydroxylation or oxidation (or both) and thereby influence qualitatively or quantitatively the metabolism of the molting hormone (or hormones).

Certainly, our present concepts of the site and mode of action of the juvenile hormones should be reexamined in light of the diversity of chemical structures which will duplicate the morphogenetic effects of the insect's own hormones. Indeed the synergists will supplement our existing tools in examining

Table 1. Morphogenetic effects of synergists and related compounds applied topically on the mealworm and milkweed bug. In the "tenebrio genitalia" test, the degrees of modification are represented numerically: 0, no effect; 1, small gin traps present or retention of short urogomphi with genitalia essentially adultoid (or both); 2, several well-developed gin traps or intermediate genitalia, or both; 3, well-developed gin traps on each abdominal segment, nearly pupal genitalia, and patches of pupal cuticle on abdomen; 4, virtually a second pupa. The milkweed bug assay scored numerically is: 1, essentially an adult with nymphal cuticle and coloration on the abdomen; 2, nymphal-adult intermediate, wings half size with nymphal coloration; 3, perfect sixth instar nymph. Both assays represent topical treatment of 20 insects per assay. Numerical assignments are based upon an 80 percent response.

Test compound	Amount applied (μg)					
	Tenebrio			Milkweed bug		
	1.0	10.0	100.0	1.0	10.0	100.0
Sesoxane	4	4	4	2	3	3
Piperonyl butoxide	0–1	2	3	2	2–3	2–3
NIA-16388	2–3	4	4	0	0	0
Sesamin	0	0	1	0	0–1	
Sesamolin	0	0		1	1	
Piperonyl farnesyl ether	0	0	3	2–3	3	3
Piperonyl farnesyl ether epoxide	0	2	3	3	3	3

this phenomenon. Clearly, very little is known about how the juvenile hormones regulate insect metamorphosis, although somewhat more is known about how synergists work. Hopefully, a collocation of knowledge from both areas will enable new insights into the mode of action of insect hormones and their structure-function relationships.

The potential of insect juvenile hormones as agents for insect control has been the subject of considerable speculation (*10*). To date, however, most of the compounds of significant activity have been available only as laboratory tools, as the cost of synthesis of these in quantities adequate for field studies has been prohibitive. The present study has revealed several compounds of high biological activity which are already available in commercial quantities.

WILLIAM S. BOWERS
Entomology Research Division, Agriculture Research Service, Beltsville, Maryland 20705

References and Notes

1. W. S. Bowers, M. J. Thompson, E. C. Uebel, *Life Sci.* **4**, 2323 (1965).
2. W. S. Bowers and M. J. Thompson, *Science* **142**, 1469 (1963).
3. W. S. Bowers, H. M. Fales, M. J. Thompson, E. C. Uebel, *ibid.* **154**, 1020 (1966).
4. Mention of a company name or a proprietary product does not necessarily imply endorsement by the U.S. Department of Agriculture.
5. K. Slama and C. M. Williams, *Proc. Nat. Acad. Sci. U.S.* **54**, 411 (1965).
6. B. P. Moore and P. S. Hewlett, *J. Sci. Food Agr.* **9**, 666 (1958).
7. H. Roller, K. H. Dahm, C. C. Sweeley, B. M. Trost, *Angew. Chem.* **79**, 190 (1967).
8. P. Schmialek, *Z. Naturforsch.* **7**, 513 (1963); H. A. Schneiderman, A. Krishnakumaran, V. G. Kulkarni, L. Friedman, *J. Insect Physiol.* **11**, 1641 (1965).
9. H. W. Dorough and J. E. Casida, *J. Agr. Food Chem.* **12**, 294 (1964); B. B. Brodie, J. R. Gillette, B. N. LaDu, *Annu. Rev. Biochem.* **27**, 427 (1958); W. W. Philleo, R. D. Schonbrod, L. C. Terriere, *J. Agr. Food Chem.* **13**, 113 (1965).
10. *World Rev. Pest Control* **3**, 4 (1964); C. M. Williams, *Sci. Amer.* **217**, 13 (1967); K. Slama and C. M. Williams, *Biol. Bull.* **130**, 235 (1966).
11. I thank Mr. J. H. Fales of the Pesticide Chemicals Research Branch, Entomology Research Division, for supplying me with the synthetic synergists employed in this study.

16 July 1968

Clay Sols versus Clay Gels: Biological Activities Compared

Abstract. *Clay gels were prepared by extrusion of sodium-Wyoming bentonite pastes through a small orifice. Clay sols were prepared from the gels by rapping on the laboratory bench for disturbance. Lettuce seeds germinated faster, microbes generated more heat, and corn seedlings absorbed more ^{22}Na in the sols than in the gels. These differences in biological activity are attributable to changes in water properties and ion activities that accompany transformation from gel to sol.*

We have tested the hypothesis that, when a clay-water system is in the sol state, biological activity differs from what it is when the system is in the gel state. This hypothesis was based on observations (*1*) suggesting that subtle changes in water structure affect biological activity, on ideas (*2*) as to how these changes exert their effect, and on laboratory evidence (*3*) indicating that changes in water structure do occur in clay-water systems during the transformation from gel to sol.

Three different experiments were performed:

1) A paste of Na-Wyoming bentonite was extruded through an 18-gauge hypodermic needle into pairs of petri dishes. One dish of each pair was subsequently rapped on the laboratory bench for disturbance of the paste within it. Then the surfaces of the pastes were smoothed with a spatula, and lettuce (*Lactuca sativa*) seeds were placed on each. The seeds were examined at intervals for determination of their rates of germination.

2) The paste of Na-Wyoming bentonite was mixed with a small amount of nutrient medium (a mixture of 0.1 percent glucose, 0.1 percent casamino acid, 0.01 percent yeast extract, and 0.01 percent arginine) and inoculated with *Streptococcus faecalis*. Immediately after inoculation the paste was extruded in equal parts into two cells of a Calvet differential microcalorimeter. One of the cells was rapped on the laboratory bench; the other was left undisturbed. The cells were then inserted in the microcalorimeter, and the difference between the heats produced by the microorganisms within them was measured.

3) The paste of Na-Wyoming bentonite was mixed with a paste of K-Wyoming bentonite for reduction of the concentration of exchangeable Na^+ to a desired level. Thereafter, sufficient NaCl was added to bring its concentration in the intermicellar solution to $10^{-4}N$, and ^{22}Na was added at a rate of approximately 0.2 μc per gram of suspension. The resultant mixture was extruded around the roots of corn (*Zea mays* L.) seedlings in glass tubes. Half of the tubes were rapped on the laboratory bench; the others were not. After 6 hours with no subsequent rapping and after 12 hours with rapping every 2 hours in a controlled-climate chamber, the seedlings from the two trials were removed from the clay, washed, and analyzed for ^{22}Na.

All results of the three experiments (Table 1) were reproducible. The results for uptake of ^{22}Na, which were amenable to statistical analyses, were different at the 1 percent level of probability. Table 1 shows that biological activity was greater in the systems that had been disturbed by rapping than in the undisturbed systems. The disturbed systems were more nearly in the sol state than were the latter; this fact was shown by several kinds of measurements. Thus our hypothesis is supported by the data.

Table 1. Effects of disturbance of a thixotropic clay-water system on biological activity within it: germination of seeds within 18 hours, heat production by microbes, and uptake of ^{22}Na by corn seedlings (cpm, counts per minute). Only the differential heat production was measured; q represents an unknown absolute value; heat production per microbe is presumed to be about 10^{-8} cal.

Seeds (%)	Heat (cal)	Uptake ($\times 10^{-3}$ cpm)	
		6 Hours	12 Hours
	System undisturbed		
74	q	5.55	7.08
	System disturbed		
98	$q+0.34$	6.28	8.75

PHILIP F. LOW
Department of Agronomy, Purdue University, Lafayette, Indiana
BRIAN G. DAVEY
Department of Soil Science, University of Sydney, Sydney, Australia
KWANG W. LEE
Department of Biochemistry, Purdue University
DALE E. BAKER
Department of Agronomy, Pennsylvania State University, University Park

References

1. W. Droste-Hansen, *Ann. N.Y. Acad. Sci.* **125**, 471 (1965).
2. P. F. Low, *Soil Sci.* **93**, 6 (1962).
3. J. H. Kolaian and P. F. Low, *Clays Clay Minerals* **9**, 71 (1962); R. A. Leonard and P. F. Low, in *Proc. Clays Clay Minerals Nat. Conf. 12th* (1964), p. 311.

13 June 1968; revised 30 July 1968