had dropped to 48 percent. Thus the genotype of blood-forming tissue alone determines the ww/WW^v difference in the ratio of red cell volume to total blood volume.

In experiment 4, the presence of 3 percent reticulocytes in blood of *ww* mice just after completion of eight daily injections of erythropoietin indicates a typical erythropoietic response under plethorization (*4*). The low percentage of reticulocytes in erythropoietin-treated plethorized WW^v mice demonstrates a defect in response to erythropoietin. However, the slight elevation of reticulocyte number between day 4 (0.1 percent, not shown in table) and day 8 (0.6 percent) is sufficiently suggestive of a minimal incomplete erythropoietic response to warrant further testing. The similar reaction of normal *ww* and anemic WW^v mice to plethorization, combined with their differing reaction to erythropoietin injection, suggests that further studies combining these treatments may yield valuable information on the cellular and biochemical basis of the delay in erythroid maturation characteristic of WW^v macrocytic anemia (*9*).

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6. These mice were produced in crosses between a *Ww* parent from the WB/Re inbred strain (maintained heterozygous for *Ww*) and a W^vw parent from a late generation backcross (b.c. 37+) to the C57BL/6J inbred strain; for convenience, they are called WB-B6F$_1$ hybrids. Experiments 1, 3, and 4 were performed at the Jackson Laboratory, experiment 2 at Blackburn College.
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8. Purified erythropoietin kindly supplied by Drs. Keighley and Lowy of the California Institute of Technology.
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Juvenile Hormone Activity: Effects of Isoprenoid and Straight-Chain Alcohols on Insects

Abstract. *A wide variety of alcohols, applied topically or injected into pupae of the yellow mealworm,* Tenebrio molitor *L., exhibit juvenile hormone activity. Included among these compounds are saturated and unsaturated alcohols with 8 to 15 carbon atoms, in straight- or branched-chains. Ether derivatives of several of these alcohols further enhance the activity.*

The juvenile hormone of insects was first demonstrated to be present in a lipid extract of the abdomens of the adult male cecropia moth, *Hyalophora cecropia* (L.) (*1*). Since this initial work, a number of biological extracts prepared from sources other than insects have been shown to possess juvenile hormone activity (*2*). Recently, several isoprenoid alcohols and their derivatives were demonstrated to possess similar activity (*3–5*). However, others (*6*) have shown that the activity of these alcohols is considerably less than that observed for semipurified extracts of *H. cecropia,* except for the methyl ether of farnesol which has been shown recently to possess several hundred times the activity of the free alcohol (*5*). We therefore attempted to determine whether these isoprenoid alcohols could be chemically modified so that their juvenile hormone activity would be enhanced or decreased. We also investigated certain other alcohols for activity.

A modification of the "*Tenebrio* test" (*4, 6*) was used in this investigation. All compounds were injected into the abdomen of newly molted pupae of the yellow mealworm, *Tenebrio molitor* L., not more than 24 hours old. Dilutions were made with purified peanut oil. Injection volumes were restricted to 0.6 μl; the solutions were injected by means of a micrometer-driven 50-μl Hamilton syringe. Two groups of six pupae were used in each test with different concentrations of the compounds. After injection the pupae were kept in small glass jars for 8 days, at which time the newly molted insects were removed and inspected under the microscope for the retention of pupal characters. The characters most sensitive to juvenile hormone activity are the pupal urogomphi and lateral abdominal gin traps (Fig. 1). The extent to which these characters were retained in the imago depended upon the activity of the compound injected. The normal adult has no gin traps, and only the male retains external remnants of the urogomphi. The greatest possible effect was retention of all pupal characters, which was achieved with one of the materials investigated; however, these insects died without further development. In most cases a positive test was signified by the retention of several gin traps and somewhat modified urogomphi (Fig. 1).

Farnesol (3,7,11-trimethyl-2,6,10-dodecatrien-1-ol), obtained commercially, was purified by column chromatography with Florisil (*7*). The fraction utilized consisted of 91 percent of the *trans trans* isomer and 9 percent of the *cis trans* isomer, as shown by comparison of the relative retention times of the four possible isomers of farnesol (*8*) in gas chromatographic analysis. All derivatives were made from farnesol so purified. Before use, other alcohols and their derivatives, examined by thin-layer and gas-liquid chromatography and infrared spectroscopy, were estimated as being more than 99 percent pure.

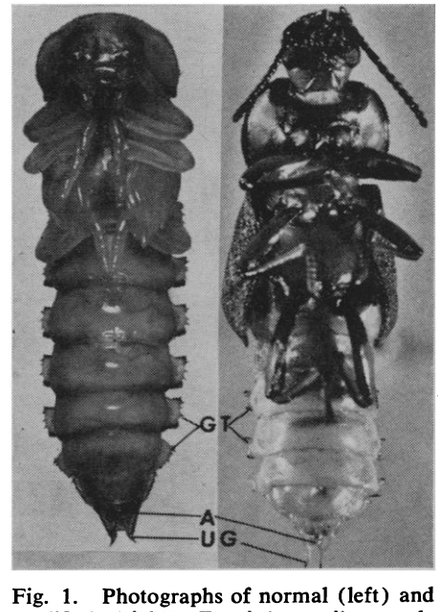

Fig. 1. Photographs of normal (left) and modified (right) *Tenebrio molitor* male beetles. In the modified beetle, the head and thorax are essentially adult, but the abdomen has pupal gin traps (*GT*), urogomphi (*UG*), and pupal cuticle on the posterior segments. The pupal aedeagus (*A*) is retained in the modified beetle.

The juvenile hormone activity of several isoprenoid and straight-chain alcohols and derivatives is shown in Table 1. The results clearly indicate the importance of unsaturation, since reduction of farnesol to the saturated alcohol decreased the activity to that of the saturated straight-chain alcohols. Removal of the hydroxyl group and further reduction to the saturated hydrocarbon eliminated activity entirely.

In the doses used, the straight-chain alcohols were only slightly active; higher doses were quite toxic. With the exception of octanyl methyl ether, all of the methyl ether derivatives were more active than the corresponding free alcohols, with the dodecanyl and tetradecanyl methyl ethers having the highest activity. The greater activity of the dodecanyl derivative is interesting in view of the fact that there are 12 carbon atoms in the farnesol carbon chain. Although dodecanyl methyl ether was slightly more active than farnesol, it was considerably less than that of farnesyl methyl ether, a situation which implies that further activity may depend on unsaturation or branching, or both.

Table 1. The effects of isoprenoid and straight-chain alcohols and some derivatives on the retention of pupal characters in *Tenebrio molitor*. The degrees of modification are represented numerically: 0, no effect; 1, Small gin traps present or retention of short urogomphi, or both, and genitalia essentially as in the adult; 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 head and thorax may have patches of adult cuticle and pigmented eyes, but the abdomen is entirely pupal. Each result represents the average amount of modification in 12 insects.

Compound	Amount injected (mg)		
	0.5	0.05	0.005
Isoprenoid alcohols			
Farnesol	3	0–2	0
Geraniol	0		
Nerolidol	3	0–1	0
Isoprenoid derivatives			
Reduced farnesol	0–2	0–1	0
Farnesane	0		
Farnesyl acetate	2–3	2	0
Farnesyltrimethyl acetate	2	0–1	0
Farnesyl oleate	0–1	0	
Farnesyl methyl ether	4	4	3–4*
Geranyl methyl ether	0–1	0	
Nerolidyl methyl ether	2–3	0–1	
Nerolidyl formate	3	0–2	0
Straight-chain alcohols			
Octanol	0–1	0	
Decanol	0–1	0	
Dodecanol	0–1	0–1	0
Straight-chain alcohol derivatives			
Octanyl methyl ether	1–2	0	
Decanyl methyl ether	2–3	0–2	0
Dodecanyl methyl ether	3	0–2	0–1
Tetradecanyl methyl ether	0–2	0–1	0

* The results of additional treatment with farnesyl methyl ether at concentrations of 0.0005 mg and 0.00005 mg were 0–1, and 0, respectively.

In previous assays, advantage was taken of the ability of corpora allata implantations, juvenile hormone extracts, and various alcohols to modify the activity of developing epidermis (*6, 9, 10*). Thus, when an active material was applied topically or injected into an insect pupa, the resulting adult, upon emergence, displayed a patch of pupal cuticle at the site of treatment. The criteria by which the cuticle was judged to be pupal included its thickness and folding, as well as its lack of adult setation and pigmentation. In the "*Tenebrio* test" the latter characteristic (lack of adult cuticular pigmentation) has been utilized as the principal criterion of juvenile hormone activity (*6, 10*). However, in our laboratory we observed that vegetable oils, extracts of different animal tissues, purified oleic acid, and mineral oil, produced unpigmented cuticular spots after injection, but had no effect on the genitalia or gin traps. Indeed, others have observed that insects treated with fatty acids may develop patches of pupal-like cuticle (*11*). It is true that extracts of *H. cecropia* do produce unpigmented white spots; however, the effect of these extracts on the gin traps and genitalia was quite pronounced and thus more easily interpreted as being due to the effects of juvenile hormone activity.

It should be noted that the effects of the compounds tested depended upon the route by which they were administered. Although the results reported here were achieved by injection, in other experiments we have found that topical treatment results in a greater degree of activity. However, the wide variations in activity among individuals in the same test relegates to topical treatment a qualitative rather than quantitative utility. Geraniol showed activity by topical application, but not by injection. Extracts of *H. cecropia* were also more effective when applied topically. But farnesyl methyl ether was an exception since it was more effective when injected.

The greater activity achieved by topical application may be due to a slow, sustained entry of the test materials into the pupa, as well as a more intimate contact with the epidermis. But the possibility that chemical reactions such as oxidation or cyclization occurred while the compound was on the surface of the insect should not be overlooked.

The information at hand is insufficient to permit any but the most general speculations about the possible structure of the natural insect juvenile hormone, if, indeed, only one exists. The current data pertaining to synthetic materials suggest that the activity is due to the presence of a hydroxyl or methoxy substituent, the latter being more active biologically and, at least theoretically, more stable chemically. Indeed, the chemical stability may contribute to the greater juvenile hormone activity possessed by the methyl ether derivative. The results of our experiments make it appear likely that the juvenile hormone, when structurally identified, will bear at least some stereochemical relationship with farnesol.

From the information presented here a potential tool in insect control may be revealed. The outstanding effect of these compounds is the deformation of the genitalia, which, if serious enough, could preclude copulation and hence reproduction. The very drastic effect on a pupa of only 5 μg of farnesol methyl ether is sufficient to warrant further investigation. The lowest dosage of this chemical that will produce anatomical sterility is not yet known. So far, we have observed only the gross external morphological effect. The effects on internal structures, which may be even more sensitive to these chemicals, await further investigation.

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