in comparison to the replica taken before the cut was made. The epidermis, however, is no longer planar; it is warped. The ridges of the long epidermal cells have become sinuate. This indicates loss in turgor and volume. The stomata were flush with the rest of the epidermis before the cut. Now they have sunk as much as 6 μm into the substomatal cavities. The most pronounced change, however, occurred in the subsidiary cells, which are immediately adjacent to the guard cells and are morphologically and physiologically distinct from the other epidermis cells. In leaves well supplied with water, these cells were bulging out up to 2 μm high. One minute and a half after the water potential in the xylem had been lowered, they have collapsed and show folds up to 1.7 μm deep (Fig. 3D). The rapidity and the large amplitude of the turgor loss in the subsidiary cells caused stomatal opening.

The epidermis responds to changes in water potential by linear displacements and distortions of the order of micrometers. Stomatal apertures are of the same order of magnitude, and since stomata are embedded in the epidermis their apertures amplify movements in the epidermis mechanically with a high gain. The stomata eventually react to the changes in water potential transmitted to them by the epidermis. The close linkage of the water potential in the epidermis to the tension in the water supply system makes stomata respond in a concerted manner and as effective regulators of water loss to the atmosphere.

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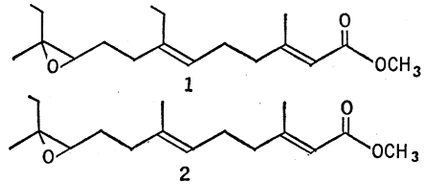
5. Supported by the Deutsche Forschungsgemeinschaft and AEC contract AT(11-1)1338. I thank H. Gobs, F. Schulz-Baldes, and E. Hartwieg for technical assistance, and the Ernst Leitz GmbH., Wetzlar, Germany, for making the interference photographs.

8 September 1969; revised 10 November 1969

Insect Juvenile Hormone: Activity of Selected Terpenoid Compounds

Abstract. *A number of compounds with terpenoid skeletons attached to various functional groups were prepared and tested for their effects as a juvenile hormone in the yellow mealworm* Tenebrio molitor. *Several of these compounds showed high activity.*

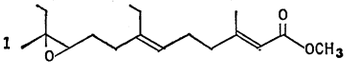
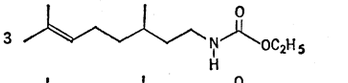
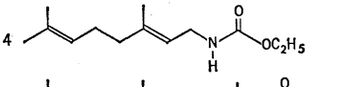
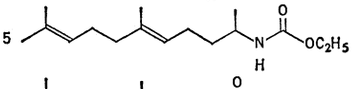
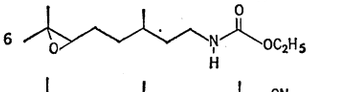
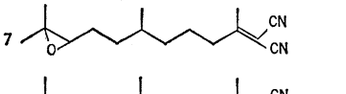
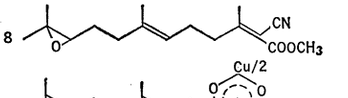
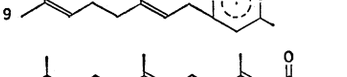
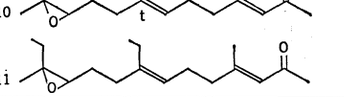
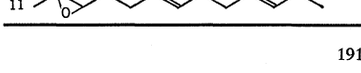
The elucidation of the structures **1** (*1*) and **2** (*2*) of two juvenile hormones from the moth *Hyalophora cecropia* (L) supports the idea that a prerequisite for compounds to display high juvenile hormone (JH) activity is the possession of an acyclic terpenoid skeleton. The most notable exceptions to this concept were reported by Bowers (*3*) who found that several nonsesquiterpenoid compounds were very active. Interestingly, replacement of a polyether side chain by terpenoid side chains markedly increased the activity of one of the compounds (*4*).



As part of our studies (*5*) of the relation of structure to activity in compounds that mimic juvenile hormones, we report the preparation and JH activity of a number of compounds (Table 1) with terpenoid backbones to which various functional groups were attached. The compounds were prepared by standard chemical methods, and their identities were firmly established by spectroscopy and analysis of elements. The carbamates **3, 4,** and **5** were prepared from the appropriate amines and ethyl chloroformate in the presence of pyridine. The amine precursors were obtained by the lithium aluminum hydride reduction of the oximes of citronellal, geranial-neral, and geranylacetone-nerylacetone, respectively. The olefinic precursors of compounds **7** and **8** were prepared by the Knoevenagel condensation of geranylacetone-nerylacetone with malononitrile and methyl cyanoacetate, respectively, with a mixture of ammonium acetate and acetic acid as the catalyst (*6*). The β-diketone of copper chelate **9** was prepared (*7*) from geranylacetone-nerylacetone, ethyl acetate, and sodium hydride. The starting material for compound **10** was the nitrile resulting from the reaction between geranylacetone, diethyl(cyanomethyl)phosphonate, and sodium hydride (*8*), which was made to react with methylmagnesium iodide to yield the desired ketone. Finally, compound **11** was prepared from the mixed isomers of methyl-7-ethyl-3,11-dimethyl-2,6,10-tridecatrienoate (*9*) by basic hydrolysis to the corresponding acid and conversion to the desired ketone with excess methyl lithium. Epoxidations were carried out with *m*-chloroperbenzoic acid. The minimum amounts of the compounds causing detectable retention of juvenile characteristics in *Tenebrio molitor* (*10*) are given in Table 1.

The activity of the carbamate compounds **3** through **6** is of interest because (i) it would appear that these are the first instances of compounds with JH activity containing nitrogen as part of the chain; and (ii) it is possible that the origin of their activity is related to the synergistic activity often found in

Table 1. Juvenile hormone activity of various compounds on *Tenebrio molitor*. Activity is the minimum weight of material causing detectable retention of juvenile characteristics when applied topically to pupae in 1 μl of acetone. Compound **1** is a mixture of all possible isomers (*9*).

Test compound	Activity (μg)
1 	0.03
3 	3
4 	3
5 	3
6 	0.1
7 	1
8 	0.1
9 	3
10 	0.03
11 	0.001

carbamates and might be of the same order as that of other synergists recently described (*3*). It is also noteworthy that no significant difference in activity between compounds **3** and **4** was observed, which is in contrast with our findings in a series of derivatives of methyl farnesate, where saturation of any double bond destroyed JH activity (*5*). Molecular size does not appear to be critical, as witnessed by the identical activity of compounds **3**, **4**, and **5**.

The synthesis of compounds **7** through **11** was undertaken in an effort to find a correlation between the electron accepting ability of the terminal functional group and JH activity. However, no such relation appears to be obvious from our results. The most interesting finding was the outstanding activity of compounds **10** and **11**. Compound **9** was as active as our synthetic mixture of isomers (*9*) of *Cecropia* JH (**1**) while compound **11**, which has the abnormal terpenoid moiety of the same hormone, showed a 30-fold increase in activity over our JH mixture.

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7 July 1969; revised 20 November 1969

Bile Pigment Formation in Plants

Abstract. *The unicellular alga* Cyanidium caldarium *evolves carbon monoxide during the syntheis of the bile pigment, phycocyanobilin. Carbon monoxide and phycocyanobilin were produced in stoichiometric amounts at comparable rates. Therefore, the mechanism of bile pigment formation in this plant parallels that in mammals.*

During the breakdown of hemoglobin in man, conversion of the porphyrin ring of heme to bile pigment is accompanied by cleavage and oxidation of the α-methyne bridge carbon to carbon monoxide (*1*). The prosthetic groups of algal biliproteins (phycocyanin and phycoerythrin) and of phytochrome in higher plants are also open-chain tetrapyrroles nearly identical to mammalian bile pigment (*2*), but little is known about the biosynthesis of these pigments. The heme precursor, δ-aminolevulinic acid (ALA), is incorporated into phycocyanobilin, the prosthetic group of phycocyanin, and the metabolic pathway for bile pigment formation in plants and animals is identical between the precursor ALA and protoporphyrin (*3*). Nevertheless, conversion of heme to phycocyanobilin by algae in vitro has not been directly demonstrated, and it is not known whether an enzymatic system is involved, similar to that recently described for the conversion of heme to bilirubin in mammalian spleen and liver (*4*). One approach to this problem would be to show that algae synthesize bile pigment and carbon monoxide concomitantly in vivo. This would suggest a similar mechanism for opening of porphyrin rings during heme degradation in plants and mammals. We now report carbon monoxide and phycocyanobilin biosynthesis in the unicellular alga *Cyanidium caldarium.*

Cyanidium caldarium cells grown in darkness lack pigment but produce phycocyanin (which contains the bile pigment prosthetic group, phycocyanobilin) when placed in light (*5*). Dark-grown cells were illuminated for 80 hours in 14 liters of nutrient medium (*5*) containing [5-C^{14}]-ALA [4.4×10^5 distintegrations per minute (dpm); specific activity 2.2×10^5 dpm/μmole] in an MF-114 New Brunswick fermentator. The suspension was aerated with 5 liters of 95 percent O_2 and 5 percent CO_2 recycled between the culture and the reservior with a circulating pump. At intervals during the illumination period, phycocyanobilin in cells was estimated spectrophotometrically by the opal glass method (*6*). At the same time, gas in the reservoir was passed through a train consisting of the following components connected in series: (i) Baralyme granules (National Cylinder Gas) to remove CO_2; (ii) Ascarite (Arthur Thomas) to remove CO_2; (iii) an alkali trap to ensure complete CO_2 removal; (iv) Hopcalite catalyst (*7*) to convert carbon monoxide to CO_2; and (v) a series of alkali traps to collect CO_2 derived catalytically from carbon monoxide. Derived CO_2 was measured with a *p*H meter by titration of residual alkali (v) to *p*H 8.5 with 1*N* HCl dispensed through a syringe microburet. The recovery of known amounts of standard carbon monoxide (Matheson) from the fermentator-reservoir-train assembly generally exceeded 95 percent. During experiments involving radioactivity, negligible amounts of radioactivity or titratable carbonate were detected in the alkali trap (iii) preceding Hopcalite in the recovery train, which indicated that CO_2 was quantitatively removed by the Baralyme and Ascarite and did not contaminate the terminal alkali traps.

Evolution of carbon monoxide (as measured titrimetrically and radiochemically) and synthesis of phycocyanobilin proceeded concomitantly in *C. caldarium* cells (Fig. 1). The cells evolved carbon monoxide only while making phycocyanobilin and not during growth in darkness or in light after cessation of phycocyanobilin synthesis. This is what one might anticipate if phycocyanobilin and carbon monoxide

Table 1. Synthesis for phycocyanobilin by *Cyanidium caldarium* cells incubated with [5-C^{14}]-ALA for 60 hours in light. Total label added to the 14 liter culture containing 140 g (fresh weight) of cells was 4.4×10^5 dpm. Theoretically, seven labeled ALA carbon atoms are incorporated into phycocyanobilin for each labeled carbon atom incorporated into carbon monoxide. The numbers in parentheses equal the total phycocyanobilin radioactivity and specific activity divided by 7, and therefore can be compared directly to the corresponding values for carbon monoxide.

Product	[5-C^{14}]-ALA incorporated (%)	Total incorporation (dpm)	Amount/culture (μmole)	Specific activity (dpm/μmole)
Carbon monoxide	0.75	3,290	70	47
Phycocyanobilin	5.15	22,650	74	307
		(3220)		(44)