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The juvenile hormones of the Cecropia silk moth
A chronicle

A. S. MEYER

Introduction

The organization committee apparently planned my talk as sort of a postprandial interlude, interspersed between this brilliant, but arduous, stuff dealing with the various Cecropia JH syntheses. So be it! I am going to present the gist of the record of how we got onto the structures of the two Cecropia JHs, and shall endeavor accurately to recount both tribulations and advances as they occurred in our work. Findings of other laboratories will be mentioned only if they directly affected our investigations, and they will be dealt with from the vantage point of the time of their publication. The account is rounded out in distinct places by some explanatory comments benefiting from the enlightenment of hindsight.

Our quest to explore the nature of JH is ineluctably rooted in Carroll Williams' discovery (1956) of the exceptional depot in adult male Cecropia moths of JH which could be readily extracted by ether and other organic solvents. This facile way of harvesting JH much impressed Howard Schneiderman at Cornell University who was engaged in studies of insect endocrinology and respiration. Subsequently he and Lawrence Gilbert (then his graduate student) prepared lipoid extracts of all kinds of natural materials and scanned them for morphogenetic activity. Sure enough, oils of some insects and crustaceans caused weak JH-like effects but, surprisingly, a few ether extracts of invertebrates other than arthropods as well as of bovine adrenals likewise exhibited morphogenetic activity (Schneiderman and Gilbert, 1959). Because none of these extracts reached the potency of the Cecropia elixir, the researchers gingerly turned to the purification of the latter. These efforts led to a 200-fold enriched preparation, the product of two consecutive countercurrent distributions of the oil (Gilbert and Schneiderman, 1961).

At that juncture it appeared to Schneiderman that the time may have come to seek professional chemical help. Through some alumni connections he approached, in 1959, the drug house where I was engaged, and its management saw in the project a perfect opening for

entering the pesticide field. Because of my long-standing interest in hormones, I was assigned to undertake the purification job, expected by some to be a shoo-in.

Bioassay and source material

In my first meeting with Howard I tried to impress upon him that success of the project might depend more upon the biological than the chemical end of the enterprise. This prediction proved itself to be fairly valid, although the chemical purification got more involved than had been anticipated originally because of some instability of the hormone structures. Such instability had not been foreseen ; for JH activity was still found in 8-year-old Cecropia specimens stored in a museum (Williams, 1958).

There were two overriding biological aspects which had to be dealt with: a reliable bioassay had to be established and the logistic of the source material had to be secured. By then Larry Gilbert had elaborated, as further part of his thesis work, a quantification of the hormonal activity (Gilbert and Schneiderman, 1960). This fine *Polyphemus* assay was, however, not much suited for routine tests in which JH activity had to be measured in numerous fractions of a purification sequence. For one thing, under normal laboratory conditions the *Polyphemus* pupae take their time to develop, as a matter of fact just about a month. Moreover, the sensitivity of a diapausing pupa towards JH changes during its aging, making the assay feasible during half a year only, from February to August, and lastly *Polyphemus* pupae were not available in unlimited numbers.

Therefore, Schneiderman proceeded in adapting his qualitative wax test (Schneiderman and Gilbert, 1959) into a quantitative assay, initially using the beetle *Tenebrio molitor* and then, to my satisfaction, switching to the waxmoth *Galleria mellonella* (Schneiderman *et al.*, 1965). Over the years, he was most ably assisted in carrying out the assays by a succession of people, notably Barbara Larson and Marjorie Boyette. These assays provided a fair estimate of JH activity in a week's time (Meyer *et al.*, 1970). It was also then that we introduced the JH unit (in some publication by Schneiderman and by Gilbert it was also variably designated as *Galleria* or Cecropia unit). The unit represents the JH activity of 1 mg of a standard Cecropia extract or, as it was later established, of a 3-ng equivalent of pure JH (*cf.* Meyer *et al.*, 1965). In the course of the many thousands of assays necessary for the JH isolation, we became aware how whimsically the test insects behaved ; their hormonal response appeared to be subject to nutritional factors, crowding conditions and other environmental effects that could not always be fully controlled. While at times fabulously accurate assay results were obtained (*e.g.* Meyer and Ax, 1965a ; Meyer *et al.*,

1970), accuracy was often less than satisfactory, though invariably the correct positive or negative responses were produced.

We availed ourselves also of the *Polyphemus* assay which was performed by Gilbert at the Northwestern University. This assay was made use of to double-check the hormonal activity of crucial fractions or to substitute for the wax test when it was not functioning for one reason or another.

It initially appeared that Cecropia material could not be collected in large enough quantities. Hence, we investigated a multitude of alternate sources. This search brought to light the fact (Table 1) that

TABLE 1

Activity of Lipoid extracts from adult Lepidoptera

Species	JHu/mg
Hyalophora cecropia	1
Samia cynthia ricini	1
Prodenia eridania	0.09
Bombyx mori	0.07
Galleria mellonella	0.03

All extracts contained hormonal activity of the same polarity.

the polarity of the JH activity from a variety of Lepidoptera was identical with that of the Cecropia hormone as judged by efficient alumina chromatography (Meyer and Ax, 1956b). Hence, it is likely that in these species the hormone structures are identical, although this result should be verified by a discriminating glc procedure. Cecropia oil was insofar more desirable than the just as potent extracts of Cynthia moths, because it contained less than half the amount of lipoidal material with similar volatility as JH. Thus we were relieved when eventually an adequate source of supply of Cecropia moths was located.

50,000-fold enriched preparation

The lack of any knowledge regarding the chemical nature of the hormone permitted the sprouting of various poorly-founded speculations. In this climate fantastic rumors made the rounds. However, when we began the purification studies in a more sober mood, only two facts had been established : (i) JH was a neutral lipoid with a defined partition coefficient and (ii) the activity of the Cecropia oil had to be enriched considerably over two hundred times to isolate the pure hormone.

At the outset, the cocoons of a large number of wild *Hyalophora cecropia* silk moths laboriously reared were collected and the animals appropriately aged ; 1,700 doomed male moths were then segregated

and their lipoid content extracted. The resulting first batch of some 300 gm of Cecropia oil was concentrated in four purification steps to yield a preparation with its hormonal potency 5×10^4-fold enriched (Fig. 1 to the extreme left). Three additional steps failed to enhance

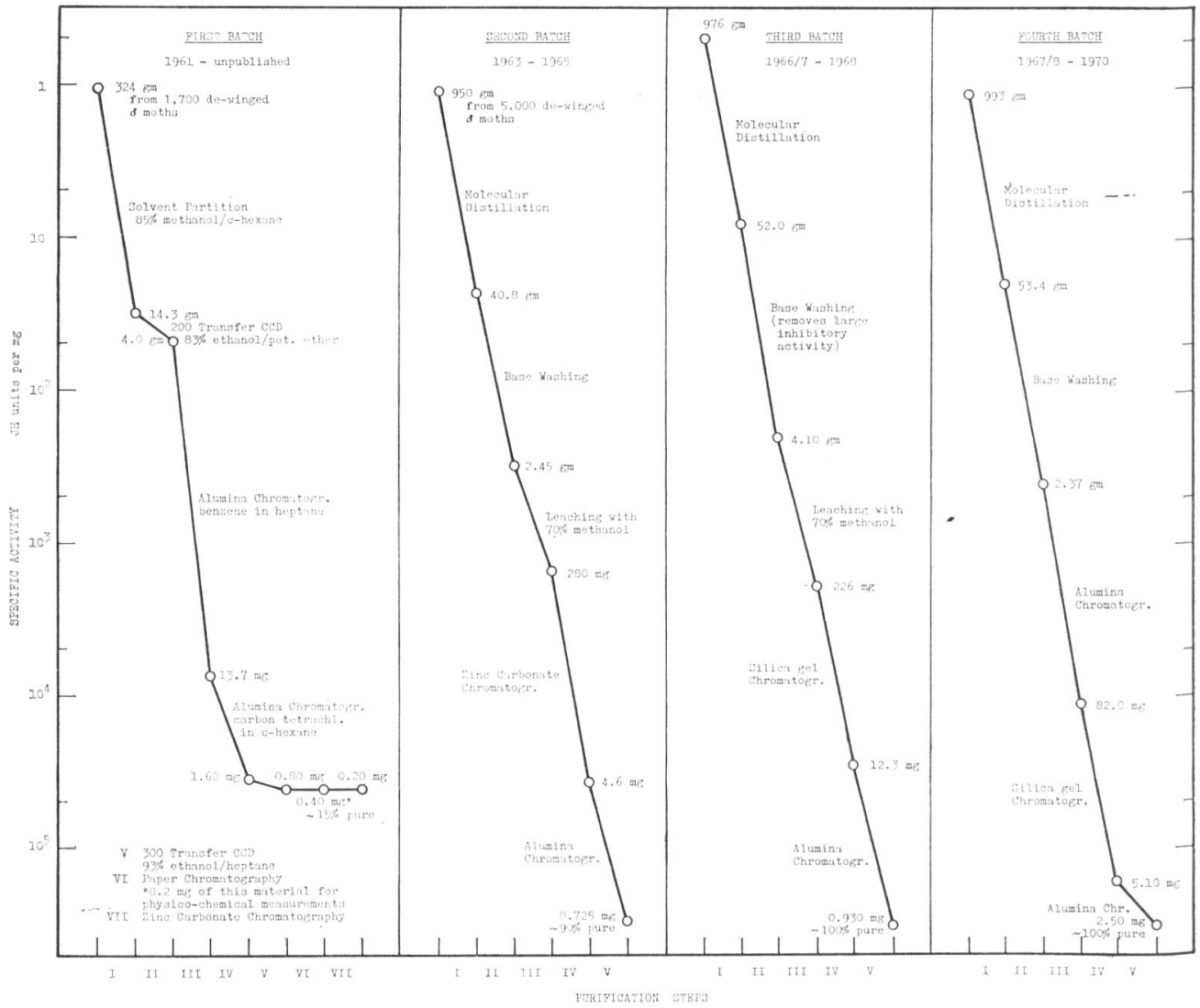


Fig. 1. — Schematic representation of the purification sequences of the four batches of Cecropia oil.

the specific biological activity of the preparation. One could have concluded from this outcome that the particular JH preparation was essentially pure. But we recoiled from such a rash verdict because in steps V and VI large losses of total hormonal activity were sustained, although the applied procedures were non-destructive to the activity of *crude* Cecropia oil. A mass spectrum of the preparation supported our doubts ; the difficulties in its interpretation led to the conjecture that the sample may have been not entirely pure, and indeed the presence

of butyl phthalate was strongly indicated. Incidentally, all our mass spectra including interpretation were obtained at the NIH-sponsored Mass Spectrometry Facility for Biomedical Research at the Massachusetts Institute of Technology through the courtesy of its director, Klaus Biemann. It was fascinating to watch this facility grow from a plain low-resolution spectrometer to an installation of unsurpassed sophistication.

But we are getting ahead of our story. By Fall 1961, subsequent to various spectral measurements of the enriched preparation, we arrived at the following conclusions : (i) Pure JH must have an activity higher than 5×10^4 units per milligram. (ii) In the mass spectrum, the largest molecular ion showed a $m/e = 294$, and it was ascribed to JH or a thermal decomposition product of JH. (iii) The most prominent feature of the uv spectrum was its intense end absorption, a result that was to mislead us at a later time. (iv) The ir spectra of various fractions were not reproducible, suggesting partial conversion of the samples while they were compressed with potassium bromide. This occurrence induced us to substitute, in future studies, a solution for the solid pellet technique. (v) At that time proton nmr microsampling techniques still required approximately 2 mg of material and were therefore beyond our reach. (vi) Countercurrent distribution experiments provided an indication (not further explored by analyses in different solvent systems or at varying temperatures) that Cecropia oil may contain an active companion compound slightly more polar than the principle constituent (Meyer and Ax, 1965a). (vii) We felt that, besides countercurrent distribution and paper chromatography, an additional analytical procedure was needed. Glc was just getting popular in the field of fatty acid analyses and seemed suitable for our purposes. It was evident, however, that the stability of the detection system of a first-generation commercial instrument would have to be improved, so that submicrogram quantities of our material could be examined reliably.

Most disconcerting were the heavy losses of biological activity of the purified fractions, especially because we were unable to pinpoint with certainty the cause of the trouble. We had a hunch that solvents might be indicated, but various control experiments left us with inconsistant results. Nonetheless, we decided to work out a *new* purification sequence employing as little solvents as possible. At the very least this approach should reduce the presence of the ubiquitous phthalate contaminants. The eventual success of the purification seemed to vindicate our presumptions. — Much later, after the individual JHs had been isolated, we were able to establish a set of conditions that allowed us to check the quality of solvents, and this is shown in Table 2. (Note that the excellent 'nanograde' solvents have been available only since about 1965.) Obviously, the requirements upon the solvents are extremely high if a few micrograms of Cecropia JH or less are to be handled without impairment. Though we suspected that the inter-

fering trace contaminants of the hydrocarbon solvents were peroxides or hydroperoxides, we were unable to prove this point (Meyer *et al.*, 1970).

TABLE 2

Recovery of methyl (±)-12,14-dihomojuvenate from solvents

Benzene, analytical reagent, redistilled	30%
» after conc. H_2SO_4 treatment	100%
Benzene, nanograde	100%
» 6 months stored (one bottle)	100%
» » » (another bottle)	37%
Cyclohexane, nanograde	100%
Hexane, nanograde	100%
Carbon tetrachloride, specially purified	100%
» » 4 months stored	90%

Hormone (1 µg) dissolved in 25 ml of solvent ; solution evaporated without delay at 10° C.

I have dwelt at some length on this, our first and indecisive experience, in order to encourage those who are going to tackle the many more insect hormones that await scrutiny not to get discouraged too early. If one makes some logical conclusions from tentative experiments and sets the course straight, one eventually must arrive at the desired end.

Nearly pure JH-preparation — two active compounds

In 1963, we finally had secured larger quantities of Cecropia material. By then, regrettably, the top management at the drug house had reversed its prior decision concerning their interest in the pesticide market, but was generous enough to accord us a reprieve of one year to come up with the JH structure. With this sword of Damocles above our heads, we undertook to process the second batch of Cecropia extract, the methods had been painstakingly elaborated in the interim. Following the five-step purification sequence, a preparation with a purity of 3×10^5 JH units/mg was obtained (Fig. 1). In the *Galleria* wax test, a few picograms of this preparation was sufficient to sustain growth, at metamorphosis, of distinct pupal cuticular tissue in the treated wound area of a waxmoth. Injection into a previously chilled *Antheraea polyphemus* pupa of as little as 5 ng of the preparation per gram of live body weight induced the test insect to develop into a complete second pupa (Meyer *et al.*, 1965).

Thanks to the help of a most gifted electronics engineer, Julius Knapp, we had augmented minimally 30-fold the sensitivity of the commercial glc unit (Knapp and Meyer, 1964 ; Meyer and Knapp, 1970), and we confidently looked forward to the analysis of the preparation we had reason to believe was highly refined. Instead of the

expected emergence of a principal peak, a dozen peaks were detected, although biological activity was restricted essentially to the two main Peaks B and E which made up approximately 75% of the total peak area (Fig. 2). The active materials were isolated and both fractions exhibited an activity of the same magnitude as had the pure preparation itself, and these were genuine potency values based on actually-weighed materials and not just on a mass estimation by comparison of glc peak areas (Meyer *et al.*, 1965).

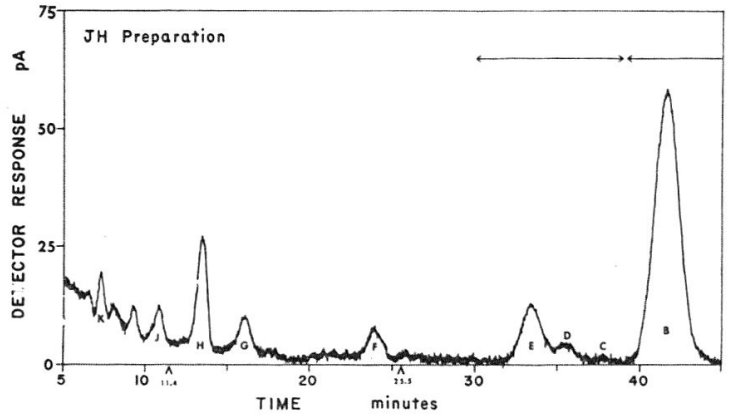


Fig. 2. — Chromatogram of JH preparation (500 ng) from second Cecropia batch after purification step V. Application directly onto packed glass column by a Hamilton ss 60 solid sampler ; quiescent current of argon detector, $I_b' = 2.5 \times 10^{-9}$ ampere. This and the following glc's were performed on neopentyl glycol adipate columns at temperatures near 175° C ; the corresponding retention times of methyl palmitate and/or methyl stearate have been marked by ∧. The double-headed arrows indicate the duration of collecting those effluent fractions that contained the hormonal activity.

When aliquots of the two active Fractions B and E were rechromatographed under the same conditions as above, similarly complex chromatograms resulted (Fig. 3). They were interpreted by grouping the peaks into three classes : (i) Peak H, appearing in the original chromatogram only and amounting to less than 10% of the total peak area, seemed to be the sole impurity present in the preparation. (ii) The 'subsidiary peaks' (A, D, F, etc.), as judged by their relative retention times were *all qualitatively identical in the three chromatograms.* This fact led us to conclude that they were artifacts of the glc system and that our preparation was nearly pure — an interpretation that is independent of the nature of the active compounds. (iii) The two, second chromatograms could be readily distinguished by the absence of Peak E in Fraction B and the absence of Peak B in Fraction E. Compounds B and E were therefore not interconvertible by glc, though their structures had to be closely related because to all appearance both compounds gave rise to the same pyrolytic decomposition products. (For further experiments concerning the pyrolytic conversion, see Meyer *et al.*, 1970.)

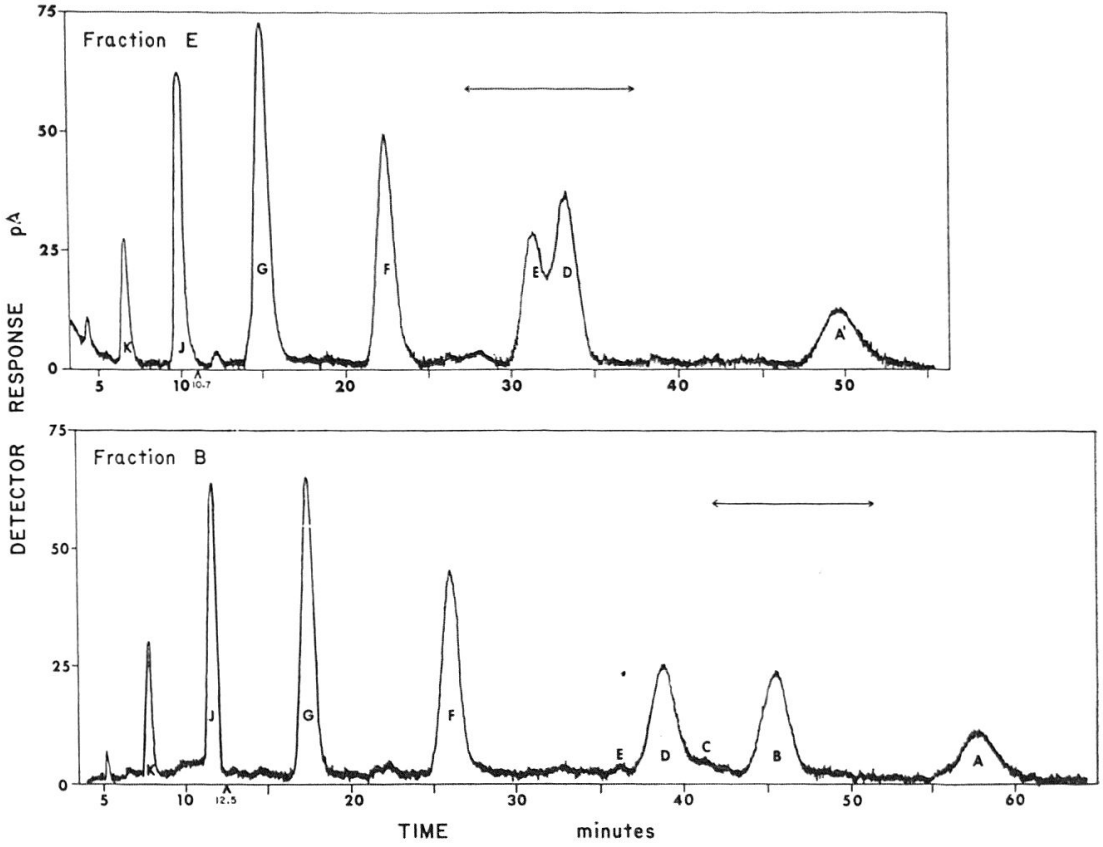


Fig. 3. — Chromatograms of Fraction E and of Fraction B (each 0.5 μg). These fractions had been isolated by micropreparative glc of the nearly pure preparation of the second Cecropia batch and trapped after passing through the argon detector at 190° C.

The question of what these active peaks represented was an agonizing one. On account of their unique biological activity it was tempting to proclaim them JH structures. But we felt that bioassay results alone were too shaky a foundation for asserting the actual isolation of two hormones, because of the uncertainties in the assays due to the often large scatter and to the presence of potentiators and inhibitors in the Cecropia extracts. Moreover, other possibilities existed such as one peak being an artifact of an earlier procedure, a metabolite of JH, or both peak being thermal decomposition products derived either from two heat-labile compounds or from a single JH. For these reasons and in view of the considerable pyrolysis encountered in our glc system (which was as good as any operated at the time), we hesitated to claim that the active peaks represented JH molecules although we left no doubt that they had to be closely related structurally to JH (Meyer *et al.*, 1965).

We had thus achieved, by the end of 1963, a JH preparation we considered to be at least 90% pure and we had demonstrated by glc that it contained two substances that possibly could (and later proved to be) JHs. That was the stand of our purification efforts when Schnei-

derman made it possible to continue the project by inviting me to join the Faculty of Biology at Western Reserve University where he then was its Chairman. Over one year elapsed until we got set-up to re-examine the samples from this second batch and we noted that, in spite of considerable precautions, the preparations were spoiled during moving and storage. This meant that we had to keep our noses to the grindstone and work-up additional Cecropia material.

Metal-free glc system

In processing the third Cecropia batch, the proven new purification sequence was altered in a minor way only (step IV). The final preparation showed again an activity of some 3×10^5 JH units/mg (Fig. 1). But its glc analysis revealed solely the two peaks that had the retention times expected for Compounds B and E (Fig. 4) and demonstrated that our JH preparation was pure (Meyer *et al.*, 1968b).

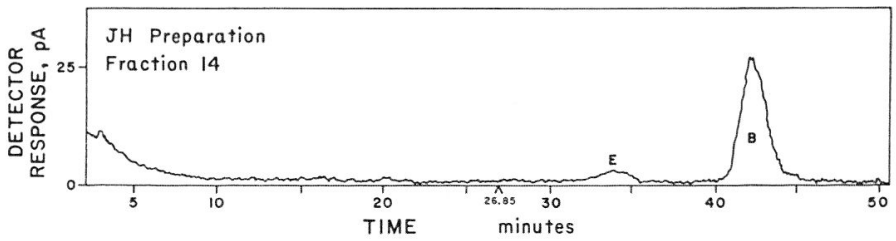


Fig. 4. — Chromatogram of a fraction of the JH preparation (81 ng) from third Cecropia batch after purification step V. Application as a solid deposit by metal-free injector. (Note increased sensitivity because of higher quiescent current of argon detector, $I_b' = 12.7\times10^{-9}$ ampere, and of solute entering the cell through the hollow tube of the anode.)

What brought about this striking simplification of the chromatogram and elimination of all those 'subsidiary peaks' we had theorized were artifacts? We had surmised that the earlier observed pyrolysis may have been induced by metal catalysis and therefore modified, in the intervening years, our glc systemso that solutes could be processed without contacting any metal surfaces. Thus we had constructed a metal-free injector, its Kel-F plunger sheltering a cavity for placement of a solute and being introduced into the system through a Teflon stopcock (Fig. 5). Furthermore, a glass effluent splitter (Fig. 6) made possible the bypassing of the brass detector cell and thereby unimpaired collection of the sensitive materials (Meyer, 1970).

In collaboration with Knapp, we also had put the detector response of argon ionization detectors on a sound scientific basis (Fig. 7) (Knapp and Meyer, 1964; Meyer and Knapp, 1970). Amongst other insights gained it was ascertained that peak areas in the linear response range were directly proportional to molar concentrations of substances

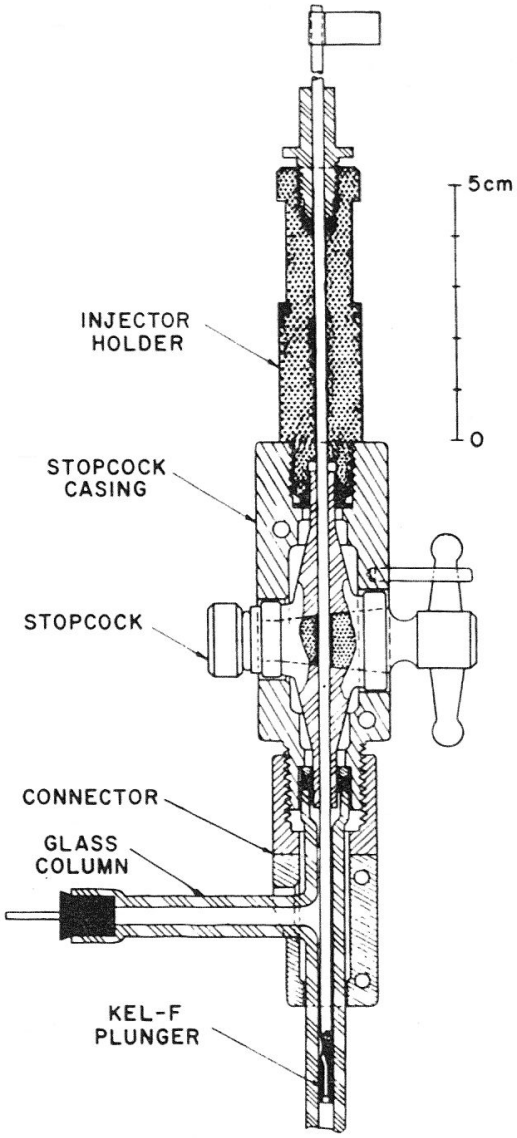


Fig. 5. — Metal-free injector assembly for glc column.

belonging to a homologous series; this allowed us to arrive at the quantitatively correct composition of the active fractions in the JH preparation. In addition we found that glassware, used in analyses of substances at the nanogram level, had better be exposed to heat, lest impurities adhering to cleanly-washed glass surfaces may obfuscate a gas chromatogram as shown for example in Figure 8 (Meyer *et al.*, 1970).

Structural aspects

Now, let us turn our attention to the structural aspects of JH. It was in 1961 that Schmialek isolated from insect material (*Tenebrio* feces) the first defined chemical of lipoid nature with morphogenetic activity and identified it as the sesquiterpene farnesol. At that time we happened to have already gathered evidence that farnesol could not possibly be identical with Cecropia JH. Amongst many available chemicals (particularly those with a terpene structure) tested for morphogenetic activity (included in footnote 46 of Schneiderman and Gilbert, 1964), we had examined farnesol and found it to be inactive. In these assays 10^3–10^4 times more material was used than necessary with our then most refined JH preparation. Subsequent to Schmialek's report, we increased the quantity of farnesol by 10^2–10^3 times and were able

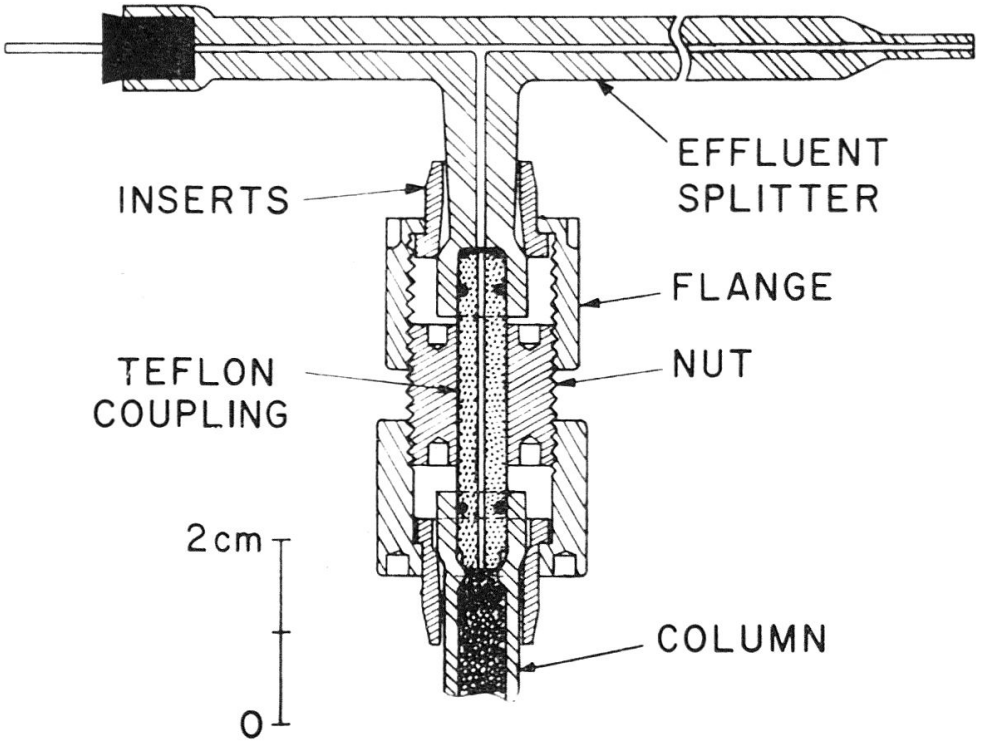


Fig. 6. — Glass effluent splitter assembly.

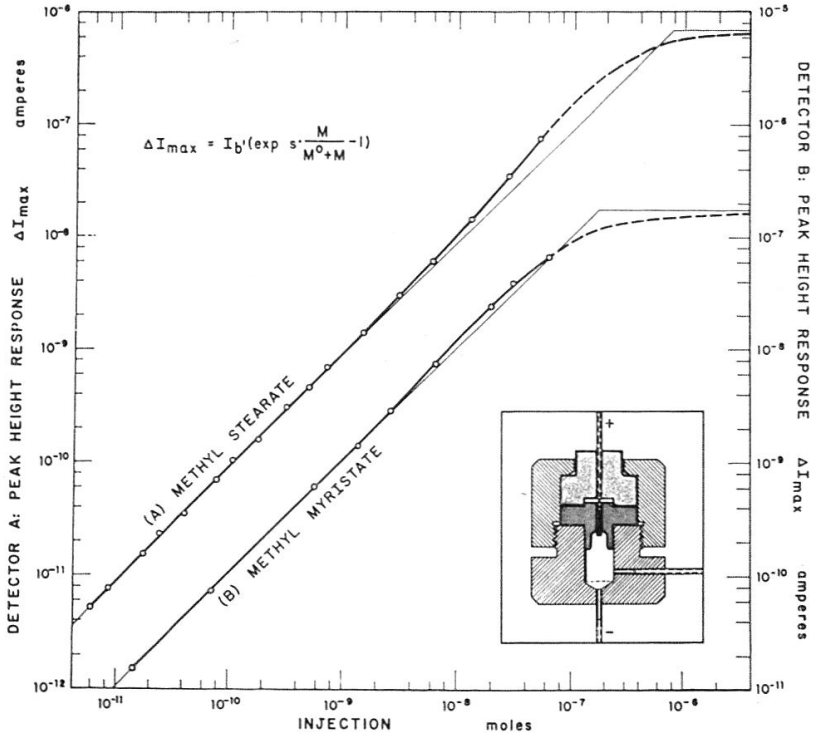


Fig. 7. — Calibration curves of small argon detectors ; materials applied onto glc columns with metal-free injector. (Note onset of deviation from linearity at generated peak height currents of approximately 3×10^{-9} ampere, corresponding under the particular experimental conditions to an injection of about 0.8 µg of a methyl ester.)

to confirm a feeble activity in the *Galleria* wax test (*cf.* Table 5, below). The sesquiterpene alcohol at these high concentrations proved, however, to be toxic in the *Polyphemus* assay and had to be injected as an aqueous ethanolic solution to produce a juvenilizing effect (*cf.* Schneiderman and Gilbert, 1964). It also seemed unlikely that the JH structure would contain an allylic hydroxyl function, because the active agent of Cecropia extract, in contrast to farnesol, did not undergo conversion on alumina chromatography. On the other hand, the hormone structure was expected to include an easily polarizable function, because JH was more tightly absorbed on silica than was cholesterol or farnesol. (Meyer and Ax, 1965b).

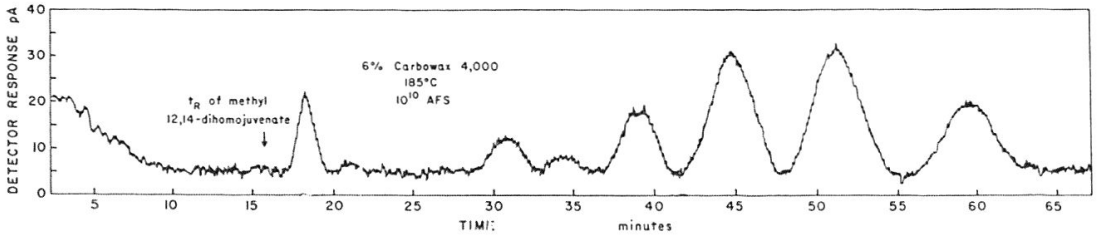


Fig. 8. — Chromatogram of impurities adhering to the surface of a thoroughly-cleaned flask. These trace impurities were collected after their slow desorption by benzene. (If the flask was heated to 200° C overnight, above impurities were absent in a chromatogram of the benzene extract.)

Trans,trans-farnesol was reported to be a biosynthetic product of mevalonate in saturniid moths (Schmialek, 1963a), but not a secretory product of the *corpora allata*, the source of JH. This was made credible by Goodfellow and Gilbert (1963) whose glc determinations yielded similar titres of farnesol in intact and allatectomized adult male Cecropia moths. Though the quantities of farnesol and JH present in the adult moth likely are of the same order of magnitude (*ca.* 1 μg), the hormonal activity exerted by the alcohol alone seems to be negligible ($<10^{-5}$).

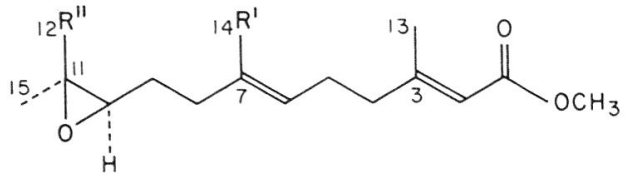
Having a model structure to guide their inspiration, chemists (in particular at Hoffmann-LaRoche) got busy synthesizing many farnesol derivatives which were tested for their morphogenetic activity (*inter al.* see Schmialek, 1963b). One of the most noteworthy compounds was farnesyl methyl ether which was remarkably effective in *Rhodnius prolixus* : injection of 24 μg of the ether per gram of live body weight — a dose that could be cut to a third with improved technique — induced a supernumerary molt of fifth-stage larvae as well as some deposition of yolk and formation of ripe eggs in decapitated adult females (Wigglesworth, 1963 ; 1969). Wigglesworth (1964, p. 296) believed that suitable formulation of the ether would cause its effects to become indistinguishable from those of the natural hormone. This finding seemed to be topped by Bowers *et al.* (1965) with the disclosure that a farnesol derivative of a higher oxidation level, the methyl 10,11-epoxy-*trans,trans*-farnesate (I), was morphogenetically 16 times more

potent ("limit of detection" 0.19 μg/mg) than farnesyl methyl ether when topically applied to *Tenebrio* pupae. At the time it was, however, quite difficult to get a valid idea about comparative activities of a certain substance in various insect species. Because farnesyl methyl ether is much less potent in insects other than *Rhodnius* larvae (*cf.* Table 6), the activity of the epoxy ester (I) in *Tenebrio* does not reach that of the ether in *Rhodnius*.

While structure I was of immediate genuine interest to us, we did not expect it to be similarly unstable as the JH we were in the process of isolating. (Indeed, the very reactions — metal-catalyzed pyrolysis and interaction with solvent impurities — that caused the losses of the minute quantities of hormone we handled, would probably not much affect bulk quantities of the material, and it is upon those that the ordinary chemical scholarship is built.) Moreover, the indication by Bowers *et al.* (1965) that morphogenetic activity of I disappeared after having been subjected to a number of the chemical reactions that also destroyed the activity of the crude Cecropia oil, failed to impress us in the absence of a more detailed description, since in our experience it was all too easy for small concentrations of morphogenetic activity to vanish on handling. We surmised also (incorrectly as it turned out) that compound I would undergo saponification under conditions that did not affect the JH-active material of the Cecropia oil. In addition and most deceiving was the apparent absence of a selective uv absorption of an enriched preparation (see above) that seemed incompatible with the presence of an α,β-unsaturated ester grouping in the JH structure. (Later, we recorded the uv spectrum of a pure sample of compound I in ether at a fourfold lower concentration (10 μg/ml) and were able to observe, superimposed onto the end absorption, a slender shoulder near 221 nm ; no wonder that it remained imperspicuous in a preparation with a content of 15% of a hormone of unknown constitution.)

Through some fateful bungling it took us 1½ years to approach Bill Bowers for a sample of his substance. By then he apparently had sent substantial quantities of it to Wisconsin (private communication, February 1970) and was able to furnish us a sample only of a stereochemical mixture containing some *cis,trans* isomer. This specimen had a weak activity in the *Galleria* wax test and was indistinguishable from that of a stereochemically uniform sample of I (*cf.* Fig. 13) that little later was kindly donated to us by Eugene van Tamelen. Since its activity in Lepidoptera was less than 10^{-3} that of JH, we were inclined to consider compound I to be not more than the foremost synthetic exploit in the series of farnesol derivatives that possibly, but not necessarily, may have a common feature with the JH structure. Despite the above presuppositions and reservations we planned to explore the reactivity of compound I, but before we had a chance to get at it, a report was published that altered our notion of the substance under discussion.

Publication of structure of "The JH"



I, $R' = R'' = CH_3$
II, $R' = CH_3$; $R'' = C_2H_5$
III, $R' = R'' = C_2H_5$

In Spring 1967, Röller *et al.* (without mentioning compound I in any way) disclosed their studies which led to structure III for the JH they isolated from Cecropia oil. Needless to describe our surprise that the hormone was a homolog of I: the methyl groups attached to the branching points at C-7 and C-11 both were extended in III by an extra carbon atom to give ethyl side chains, a novel feature in terpene chemistry. The structural deductions presented in their first communication were not complete (a fuller account was published by Trost in April 1970), and this may have lain at the root of the skepticism by which their proposal was initially received in certain research circles. Thus, for instance, the circumstance that mevalonic acid seemed not to be incorporated with ease into JH or that epoxidases were not readily found in insect tissues led to questioning of the structure. But to us, the proposed constitution appeared reasonable especially because the reported values of the mass spectrum corresponded to those we had obtained from the enriched preparation of the first batch of Cecropia oil. We appreciated the great strides made by the elucidation of the first JH structure. Already in the Fall of the same year the Wisconsin group reported the synthesis of JH and established thereby the stereochemistry of the molecule as shown in the above formula (Dahm *et al.*, 1967). Now, there could be no longer the slightest doubt as to the structure of the isolated hormone.

On the other hand, the designation of III as "The JH" was ill-advised and reflected the then propounded concept of that group of researchers. Even without this bias it would have been not easy for them to detect the presence of the second morphogenetically active substance in Cecropia oil, because of their choice of a coleopterous test insect, the *Tenebrio* mealworm, for assessing the hormonal activity of a lepidopterous extract. Later our bioassay revealed that both substances were similarly potent in Lepidoptera and other insect species, but the less abundant constituent was up to tenfold less active in *Tenebrio* pupae (*cf.* Johnson *et al.*, 1969). Moreover, inclusion in their purification scheme of a temperature-programmed glc as the terminal

step, though yielding a 300 or 50-fold enrichment of the hormonal activity (see Fig. 9), was another important difference in their technique not conducive to make recognition of "Compound E" any easier. Only after the work-up of their second Cecropia batch was published early in 1969 (Röller and Dahm, 1968), was it possible for us to be sure that the respective pure preparations of both laboratories indeed reached identical morphogenetic potency, notwithstanding the fact that substantially different purification sequences and assay procedures had been employed (Fig. 9).

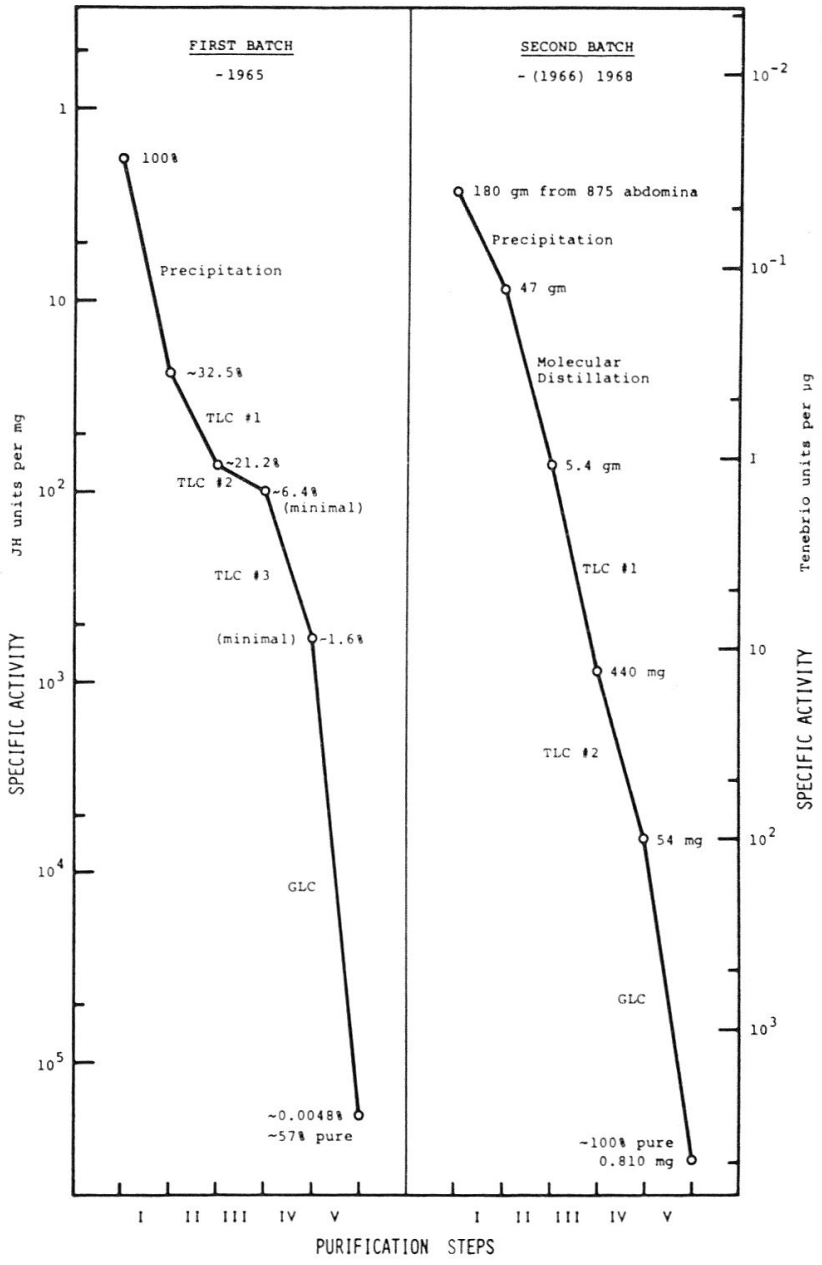


Fig. 9. — Schematic representation of the two purification sequences of Cecropia oil reported by Röller *et al.* (1965, 1968).

Identity of Compound B with Röller's JH
Structure of Compound E: less abundant Cecropia JH

When the news concerning the JH structure broke, we had established that the more volatile Compound E was also slightly more polar than Compound B and that the latter compound remained unaffected on glc (Meyer *et al.*, 1968b). This allowed us to rule out some possibilities (see above) with regard to the significance of these substances. We also had started with the spectrometric examination of the pure JH preparation from the third Cecropia batch. Furthermore, upon microhydrogenation of a fraction of the pure JH preparation we isolated three conversion products that were subsequently identified by spectrometry (Meyer *et al.*, 1968b ; 1970). Accordingly, Compound B was found to have structure III which was thereby confirmed. With the publication of the proton nmr data of the synthetic JH by Dahm *et al.* (1967) in which the small calibration error of the prior report was corrected, the last vestigial discrepancy in data was removed and there could be no more question that our Compound B was identical to their JH (III).

We had, however, still a job to do. What about the second substance with morphogenetic activity, our Compound E? We could not rule out that it might be an artifact (*e.g.* β,γ-unsaturated ester) from an early step in the purification sequence. But a more attractive hypothesis was to consider Compound E a lower homolog of III. However, an argument against this assumption could be made on comparing the retention ratios of the two active compounds in glc (E : III and I : E should be exactly the same to satisfy a straight homology relationship, but see Table 3). Furthermore, it seemed strange that pyrolysis of

TABLE 3

Retention ratios in glc

Methyl esters	Adjusted retention times (min)	Ratio of their log.
12-homo : 12,14-dihomo juvenate . . .	11.26 : 13.06	1 : 1.06
juvenate : 12-homo juvenate	8.70 : 11.26	1 : 1.12
juvenate : 14-homo juvenate	8.70 : 10.12	1 : 1.07

On Carbowax 4000 at 195° C.

two homologous compounds should lead to apparently identical, and not homologous decomposition products (see above).

We proceeded to separate the two components of our pure JH preparation by micropreparative glc and were able to trap a total of 65 µg

of Compound E. This material was carefully assessed as to its homogeneity and then subjected to various spectroscopic measurements. First we recorded the proton nmr spectrum of pure Compound E and found it in many respects identical with the spectra of I and III, manifesting identical stereochemistry of the three compounds. The differences (Fig. 10) readily convinced us that Compound E was the 12-homo analog of I or for that matter the 14-nor analog of III. Thus the triplet absorption near δ 0.99 ppm, deriving from the methyl protons in the two ethyl side chains at C-7 and C-11 of III, was only

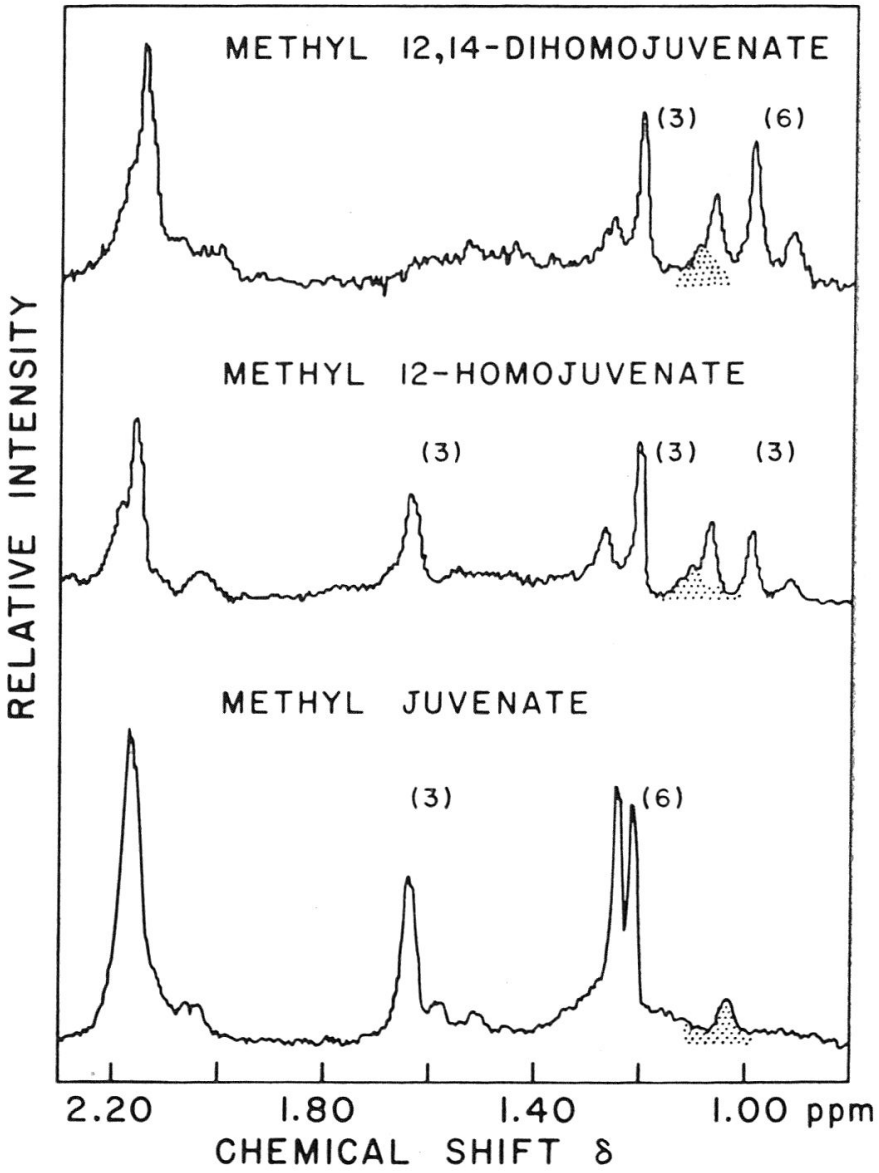


Fig. 10. — High-field segments of proton nmr spectra of synthetic compound I and natural compounds II and III in carbon tetrachloride. Values in parentheses are relative peak areas in round figures ; stippled zones are due to solvent impurities.

half as intense in the spectrum of Compound E and indicated shortening or loss of one side chain. To be sure, a new singlet accounting for three protons appeared in the spectrum of Compound E at δ 1.63 ppm, a peak familiar from the spectrum of I and ascribed to the *trans*-allylic methyl group at C-7. In I the second ethyl group, attached to the oxirane ring at C-11 of III as well as of Compound E, is also replaced by a methyl group as evidenced by the double peak near δ 1.2 ppm and the absence of any resonance near δ 0.99 ppm. Our contention that structure II had to be assigned to Compound E was supported in every detail when the ir spectrum (Fig. 11) and the high-resolution

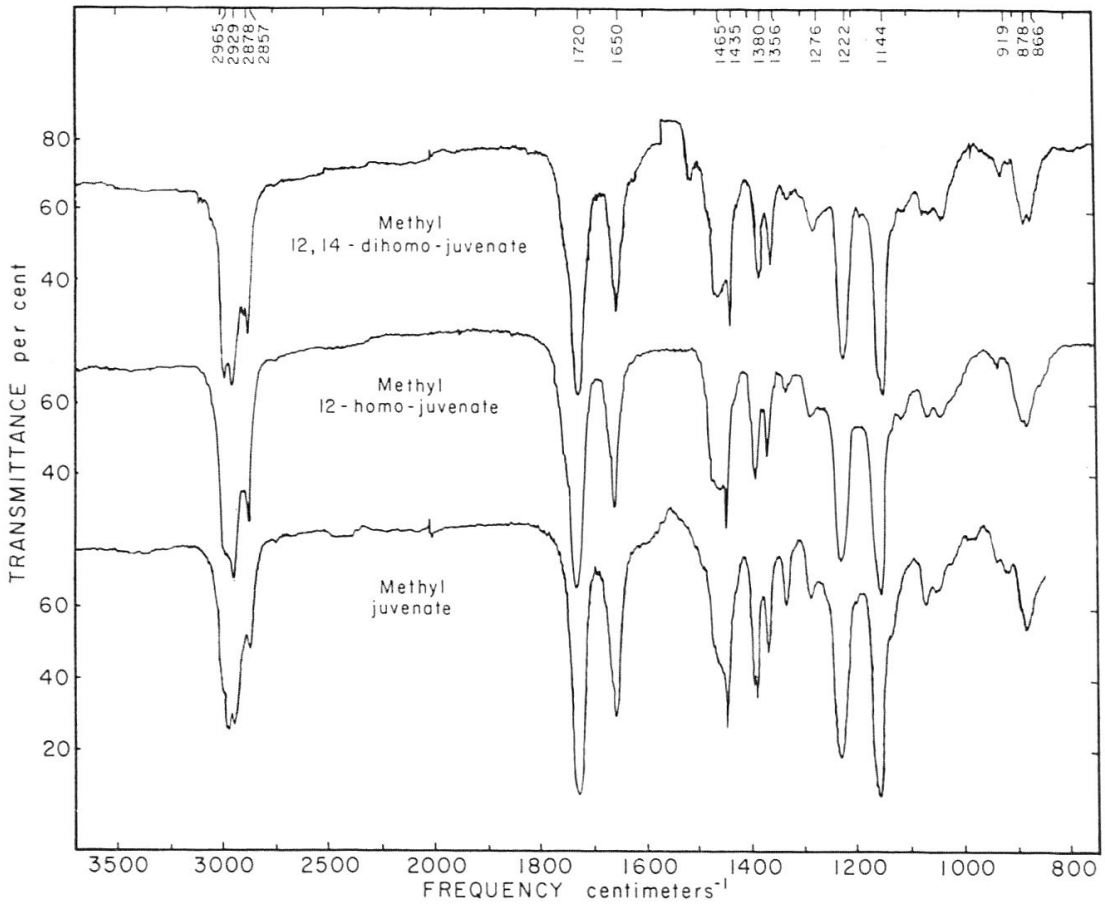


Fig. 11. — High-resolution ir spectra of synthetic compound I and natural compounds II and III in carbon tetrachloride.

mass spectrum (Fig. 12) of Compound E as well as the mass spectrum of its tetrahydro derivative were compared with the corresponding spectra of I and III. These data have been discussed elsewhere (Meyer *et al.*, 1970). As a result the structure of Compound E (II) was unequivocally established, and we believed it reasonable to conclude that this natural product was a second Cecropia hormone. Depending on the

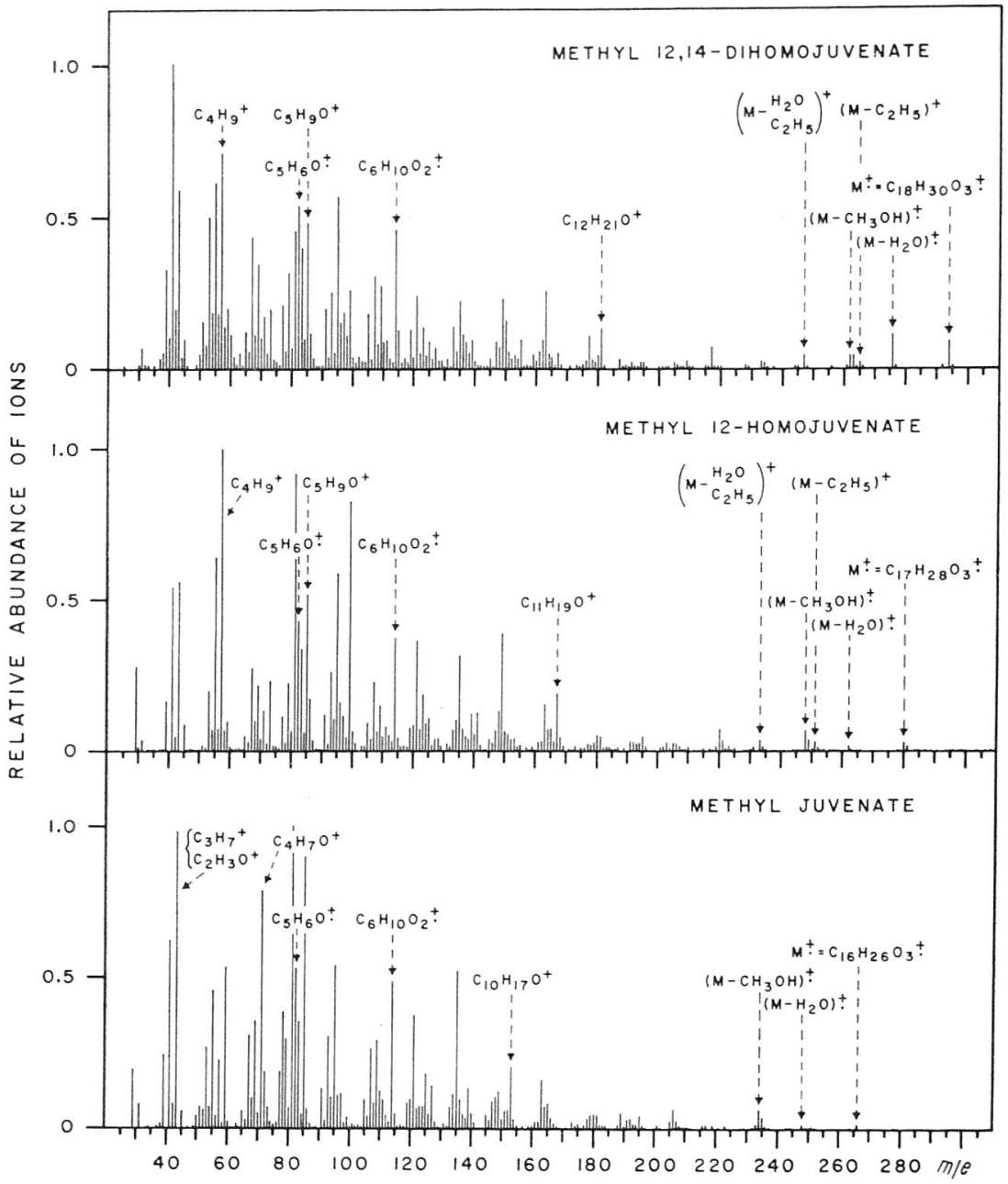


Fig. 12. — Mass spectra of synthetic compound I and natural compounds II and III. Elemental compositions were determined by high-resolution mass spectrometry and have been indicated in the diagrams for a number of selected ions ; the abundance of these ions amounts to 75% or more of that represented by the particular bar.

individual Cecropia batch, II was responsible for 13 or 20% of the endocrine activity.

The deviation of the glc retention ratio of II from a straight homology relationship with I and III had to be rationalized by some inductive effect of the ethyl group upon the adjoining oxirane function that caused it to become slightly more polar. No such effect was observed

when the methyl group attached to the olefinic linkage in the center of the molecule is lengthened to an ethyl group (I : methyl (±)-14-homojuvenate, see Table 3) (*cf.* Mori *et al.*, 1969). We also undertook a search for the possible presence in Cecropia oil of compound I or of the *trans*-10 isomers of the JHs, but we were unable to detect any trace of these compounds (Meyer *et al.*, 1970). If mevalonate were a biosynthetic precursor of the Cecropia JHs (which has by no means been conclusively ruled out), one might have expected to find probably some methyl juvenate in the extracts ; since this is not the case, it appears worthwhile to take a closer look at the alternate possibility of a homomevalonate precursor (as suggested first by Meyer *et al.*, 1968a).

Nomenclature

With the finding of a second JH a problem of nomenclature arose. Compound I seemed a fair candidate to be given a common name that would aid in conveniently distinguishing the two Cecropia JHs as well as their analogs and stereoisomers. We communicated our thoughts to van Tamelen who first had synthesized the compound. He shared our concern and proposed methyl juvenate as an evocative trivial name for I. We have since shown how this notation can be applied in a semisystematic manner (Meyer *et al.*, 1970) and would like to urge the members of this assembly to end the incoherent and confusing nomen-

TABLE 4

Glossary of terms in usage for JHs and substances with "JH-like activity"

Abstruse	Correct	Recommended
The JH or Cecropia JH	Cecropia C_{18}-JH or JH-1	Methyl 12,14-dihomojuvenate or Methyl 7′,11′-dihomojuvenate (III)
Methyl JH or C_{16}-JH	Cecropia C_{17}-JH or JH-2	Methyl 12-homojuvenate or Methyl 11′-homojuvenate (II)
Synthetic JH	Methyl 10,11-epoxy-*t,t*-farnesate	Methyl juvenate (I)
Epoxy C_{17} ethyl ester		Ethyl 12,14-dihomojuvenate
Position isomer of Cecropia C_{17}-JH		Methyl 14-homojuvenate
t,t,t C_{17} methyl ester JH isomer		Methyl 12,14-dihomo-*trans*-10-juvenate
C_{17} methyl ester		Methyl 12,14-dihomo-*trans,trans-cis*-farnesate
Synthetic JH mixture	Law *et al's* (JH) mixture	Hydrochlorination product according to Law *et al.*
Analog (activity)	Bio-analog or Juvenoid	(Hormono)mimetic or morphogenetic compound
		Analog (structure)

clature that has arisen in the JH field by adopting this proposal having structural connotations. In Table 4 we have exemplified the present dilemma and let the glossary speak for itself. Berkhof (1970) also has drawn attention to a nomenclature abuse and we share his views as far as they go.

Are the Cecropia JHs racemic products or single enantiomers?

In late 1967 we happened to hear of Bill Johnson's progressing efforts in synthesizing methyl (±)-12,14-dihomojuvenate (III) by a stereoselective procedure (Johnson *et al.*, 1968). We told him about the second JH and were most gratified when he showed interest in preparing methyl (±)-12-homojuvenate (II) by his elegant and efficient method (Johnson *et al.*, 1969). The synthesis confirmed our structure assignment based on spectral data.

When this synthetic material was evaluated in the *Galleria* wax test, it showed in repeated assays a tendency toward a lower bioactivity than the natural hormone, but it was not possible to distinguish with certainty the potency of the two preparations (Meyer *et al.*, 1970). In Figure 13 such a comparison is illustrated. (A bioassay of compound I

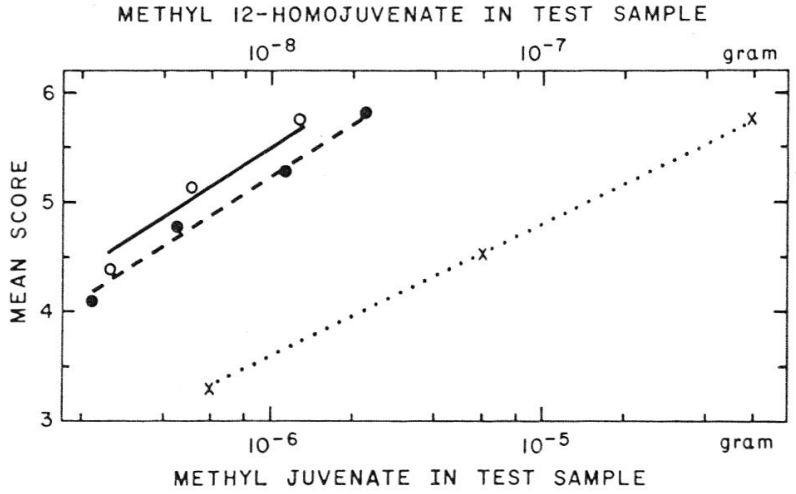


Fig. 13. — *Galleria* wax test of homogeneous natural (o———o) and racemic synthetic (•- - - -•) methyl 12-hormojuvenate (II) as well as of synthetic methyl (±)-juvenate (I) (x· · · · ·x).

has been included in the graph.) From bioassay data alone it was thus not possible to ascertain that the natural hormones are single enantiomers. (*Added in proof:* V. B. Wigglesworth informed me about the outcome of an evaluation of various samples, dissolved in peanut oil, in his topical *Rhodnius* assay (1969). In a carefully conducted test series, the natural methyl 12,14-dihomojuvenate (III) (containing

9 mol% of II) proved to be about twice as potent as the synthetic racemic product III of Johnson *et al.*, 1968 ; *cf.* also Table 5.)

Recently we measured the optical rotation of a fraction of pure JH preparation from our fourth Cecropia batch. The fact that it exhibited a plain positive dispersion curve (Fig. 14) proved that the Cecropia JHs are indeed enantiomers (Meyer and Hanzmann, 1970). Thus, the systematic name of methyl 12-homojuvenate for instance is (+)-*cis*-10,11-epoxy-3,7,11-trimethyl-*trans*,*trans*-2,6-tridecadienoate (II). At present, however, it would be speculative to assign the absolute configuration to the JHs on the basis of these data and rotation values of the few epoxides of known configuration, but we have initiated experiments that should provide the desired information. (*Added in proof:* The chiral centers of methyl 12,14-dihomojuvenate (III) have been established to have 10*R*,11*S* configuration. Meyer, Hanzmann, and Murphy, in press.)

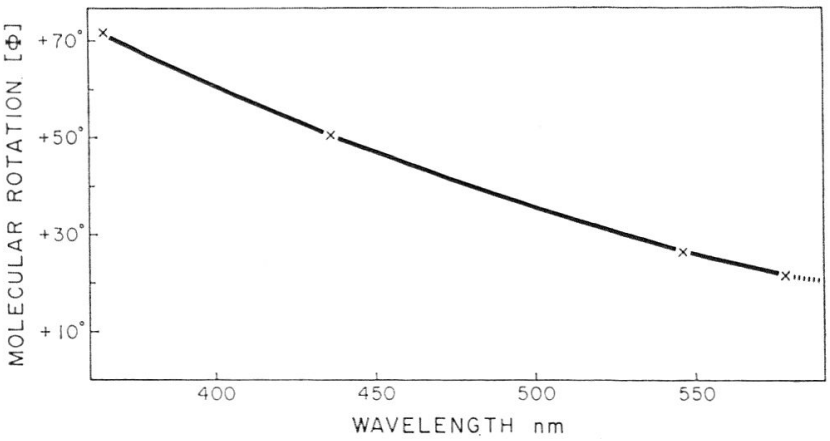


Fig. 14. — Optical rotatory dispersion curve of pure JH preparation in chloroform at 28° C.

Biological data

In the remaining time I wish to talk about some biological work with which I have been associated. JH application reproduces the effects of *corpora allata* implantation in insects. The minimal doses of methyl 12,14-dihomojuvenate (III) that block development in a variety of species were determined in collaboration with A. Krishnakumaran and are listed in Table 5 (Meyer *et al.*, 1970). Since a JH effect is dependent on a variety of parameters such as timing, sensitivity of the tissues, chemical and enzymic inactivation of the hormone, it is interesting to note that a single injection of *ca.* 2 µg/gm of JH at an appropriate time induces development of a complete supernumerary stage in *H. cecropia*, and *Galleria*, as well as in *Rhodnius*. Polyphemus pupae previously chilled respond to a much smaller

quantity of the hormone, and they therefore have been initially favored as test insects. On the other hand, *Tenebrio* pupae and *Pyrrhocoris apterus* larvae require high doses of Cecropia hormone (and compounds more effective than the hormones have been synthesized) ; this fact may encourage the search for additonal natural JHs.

TABLE 5

Development of a supernumerary stage : Minimal dose of methyl 12,14-dihomojuvenate

Species and Stage		Weight gm	Injection Dose µg/gm	Topical Dose µg/gm	Reference
A. polyphemus	pupa	4.0	0.005 (chilled)		1965
H. cecropia	pupa	4.0	1.2 (unchilled)		1969 *
Galleria	pupa	0.11	2.5		1970 *
Galleria	larva	0.16	1.9		1968
Tenebrio	pupa	0.12	90.	10.	1970 *
Rhodnius	larva	0.13	2.3	1.5 †	Wiggles-worth *
Pyrrhocoris	larva	0.020		~250.	Bowers *

* Assay performed with synthetic racemic III.

† Private communication.

In Table 6 the morphogenetic potency of a number of compounds mentioned earlier in the paper are compared with the activity of methyl 12,14-dihomojuvenate. In all three species tabulated the Cecropia JHs

TABLE 6

Relative doses in topical assays evaluating morphogenetic effects

Methyl juvenate		*Galleria* [a]	*Tenebrio*	*Rhodnius* [c]
12,14-dihomo	(III)	1	1[b]	1
12-homo	(II)	1	4[b]	1.5 †
Parent compound	(I)	10^3	(20)	40 *
Farnesyl methyl ether		10^5	(3×10^2)	4 (!)
Trans,trans-farnesol		10^6	(3×10^4)	400
Dodecyl methyl ether		10^5	(3×10^4)	$>10^3$

[a] Meyer *et al.*, 1965, 1970 ; Schneiderman *et al.*, 1965.

[b] Johnson, Krishnakumaran *et al.*, 1969.

[c] Wigglesworth, 1969 ; and † private communication. * Ethyl ester.

() From various reports, including Bowers *et al.*, 1965.

are the most effective compounds of those listed. While both hormone molecules seem to be similarly active in many species, a significant difference of II and III has been discerned in *Tenebrio*. It is also obvious that the three species vary widely in their specificity requirements towards various structures. That farnesyl methyl ether is unusually potent in *Rhodnius* has been mentioned earlier. Many more compounds with morphogenetic activity have been reported and were referred to elsewhere (*cf.* Meyer *et al.*, 1970). Whereas all these substances induce notable morphogenetic changes, it is not clear which ones react initially with the same receptors as do the JHs. The time may be near when it will be possible to sort out the various compounds according to their primary target, learn more about the way they interfere with an insect's metabolism, and bring some order into the plethora of structures with morphogenetic activity.

Corpora allata involvement is however not limited to morphogenesis, nor are the effects of JH. Pierre Joly found in *Locusta migratoria* larvae that the Cecropia hormones exert their chromatotropic effect shortly after the period during which the morphogenetic determination of the larvae is realized. Moreover, injection of the hormones into female locusts that have been deprived of their *corpora allata* immediately after metamorphosis, restores their normal ovarian development. Thus, the same agent is responsible for a variety of actions presumably by combining with receptors that are sensitive at various times of the insect's life cycle (Joly and Meyer, 1970). Franz Engelmann assayed some samples on allatectomized female *Leucophaea maderae* roaches (*cf.* Engelmann, 1969). He concluded (private communication) that methyl (±)-12,14-dihomo, (±)-12-homo, and (±)-14-homojuvenate all stimulate at similar rates synthesis of the female specific hemolymph protein necessary for egg maturation. The *cis*-6,*trans*-10 isomer of racemic III (van Tamelen and McCormick, 1970), in contrast, is inactive and may profitably be studied as to its possible inhibitory properties. Krishnakumaran showed that methyl (±)-12,14-dihomo (III) and (±)-12-homojuvenate (II) are both similarly effective in terminating the diapause of non-chilled debrained saturniid pupae (Johnson *et al.*, 1969).

Regulation of development

In conclusion, I would like you to take a look at an experiment in which varied quantities of Cecropia JH were injected into last instar *Galleria* larvae of different age, a study on morphogenesis done by František Sehnal during his stay in Cleveland (Fig. 15) (Sehnal and Meyer, 1968). If injection of adequate amounts of the hormone takes place on day 4 of the particular stage or later, intermediate larval–pupal forms develop, because some tissues of the moth have lost their capacity to respond to JH at this advanced age; the emerging intermediate forms can be considered to be pathogenic oddities.

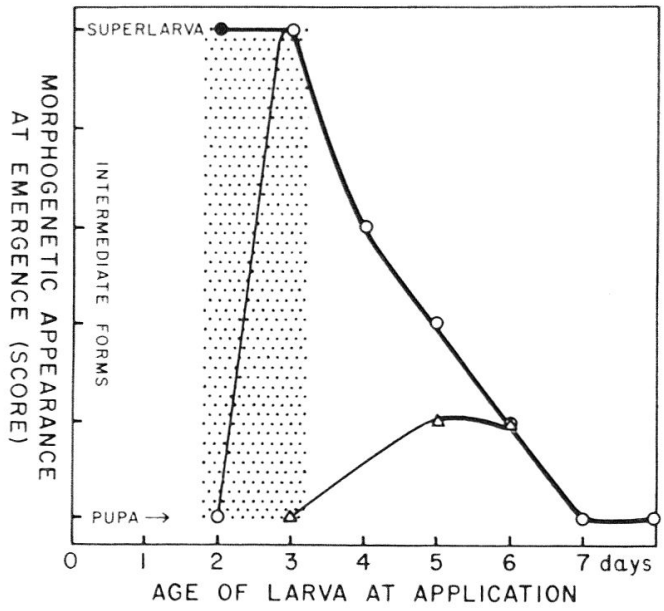


Fig. 15. — Graphic representation of varying morphogenetic effect of Cecropia JH with advancing age of last instar *Galleria* larvae. △ Single injection of 0.15-μg equivalent of JH at specified day ; O single injection of 0.3-μg equivalent of JH at specified day ; ● injections of 0.3-μg equivalent of JH each on days 2 and 4. Stippled area indicates early period of JH sensitivity in waxmoth.

More important is the physiological response that can be duplicated by a single injection of 0.3 μg of Cecropia JH on day 3 or of a higher dose on day 2. None of the relevant tissues has by then ceased to be sensitive to the hormone. In this case we observe an all or nothing effect — either superlarvae or pupae result, but never intermediate forms. The end result depends upon whether or not the JH level is sufficiently high to be effective throughout the *entire period of JH sensitivity* of the insect as a whole. Apparently, as long as any cells of the insect body are still sensitive to JH, no prior change caused by the hormone is fixed. The idea of sets of special genes that coordinate the JH effects in various tissues and thereby ensure an orderly progression of the development presents itself. Such genes would have to directly or indirectly respond to JH at the end of the sensitive period of a particular stage, and in turn cause earlier signals received by the genome to be validated or invalidated. The *Galleria* system might be as good as any to ascertain whether or not some extragenetic mechanisms are implicated in these processes.

By answering such basic questions, the research on JH is going to significantly contribute to the solution of developmental problems. One can predict with confidence, however, that the regulation will be more complex than presently fathomed, and hormones and neurohormones that await their isolation or even their recognition will be involved in one way or another. Nonetheless, it seems unlikely that JH

will be dethroned from the central role it has been assigned to play in these momentous proceedings that usher in, at the propitious time, the fulfillment of an insects's foreordained life plan.

Acknowledgements

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