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## Chemistry of Natural Products with Juvenile Hormone Activity

M. JACOBSON

Extracts with juvenile hormone (JH) activity were first prepared from the abdomens of male *Cecropia* moths by Carroll Williams in 1956 (1). In the following two years, Schneiderman and Gilbert (2, 3) reported that they had been able to prepare JH active extracts from adults, eggs, larvae, or pupae of insects from several orders as well as from a number of invertebrates. They stated (4) and I quote "... we are dealing with a group of substances which are widespread in Nature, but are probably not universal". In 1960, these investigators (4) reported JH activity in extracts of 6 species of microorganisms out of 29 tested, as well as in ether extracts of soybean meal. The activity of all of these substances was far below that of *Cecropia* and the active components were not isolated.

In 1961, Williams (5) obtained a patent covering active extracts of *Cecropia* prepared with ethyl ether, petroleum ether, methanol, and ethanol, but it was not until 1965 that one of the active components was isolated by Röller and coworkers (6) and another two years before it was identified (7). The second active *Cecropia* component was identified in 1968 by Meyer and coworkers (8).

As early as 1959 Karlson and Schmialek (9) reported JH activity in the feces of the yellow mealworm, *Tenebrio molitor*; the active components were isolated in pure form and identified as farnesol and farnesal by Schmialek (10) in 1961. Here, also, the activity of these compounds in the pure state was far below that of pure *Cecropia* juvenile hormones.

The presence of substances with high JH activity in the wood of certain evergreen trees, especially in balsam fir (*Abies balsamea*), was reported in 1965 and 1966 by Sláma and Williams (11). The active component juvabione (or "paper factor") was identified in 1966 by Bowers and coworkers (12), and another active component, dehydrojuvabione, was identified in 1967 by Cerny and coworkers (13). Whereas, the *Cecropia* JH is active by injection or topical application in a rather broad spectrum of insect species, juvabione and dehydrojuvabione appear to be active only in a few species of the family Pyrrhocoridae.

Subsequently, Staal (14) in 1967 surveyed a large number of conifers for JH activity by exposing *Dysdercus volkeri* larvae to filter paper discs impregnated with methanol extracts of the shoots and leaves. Results were almost entirely negative, only trace activity being shown by extracts of some species of *Cephalotaxus*. At the 1968 Conference on Insect-Plant Interactions held in Santa Barbara, California, Bowers (see Williams and Robbins, 15) reported that among 52 species of plants tested for JH activity on *Tenebrio*, 6 gave active extracts, of which 2 showed considerable activity.

Wellington (16), in 1969, found that ether extracts of the wood of Douglas fir and red cedar applied topically to the larvae of the forest tent caterpillar (*Malacosoma pluviale*) and *Tenebrio molitor* produced morphological abnormalities typical of JH. On the basis of this work, Mansingh and coworkers (17) in Canada in 1970 assayed wood and bark extracts of 5 trees for JH activity. Methanol-dichloroethane extracts of the plant parts were assayed topically on pupae of the wax moth, *Galleria mellonella*, by application to a wound made on the thoracic dorsum. Activity response of the extracts was arbitrarily divided into 5 numerical ratings ranging from 0 (no activity) to 4 (highest activity). As can be seen in Table I, although some activity

TABLE I  
*Bioassay of juvenile hormone activity of wood and bark  
extracts of several trees*

Extracts	Activity response *					Activity Index
	0	1	2	3	4	
Farnesyl methyl ether . . . . .	0	4	3	5	2	2.36
Balsam wood . . . . .	6	6	4	4	0	1.30
Cedar wood . . . . .	7	8	2	1	0	0.83
Spruce wood . . . . .	6	4	0	0	0	0.40
Hemlock wood . . . . .	11	7	0	0	0	0.39
Pine wood . . . . .	12	4	0	0	0	0.25
Pine bark . . . . .	4	8	0	0	0	0.67
Cedar bark . . . . .	8	0	2	0	0	0.40
Spruce bark . . . . .	7	2	1	0	0	0.40
Hemlock bark . . . . .	7	3	0	0	0	0.30
Balsam bark . . . . .	9	1	0	0	0	0.10
Control . . . . .	25	0	0	0	0	0

\* No. of individuals per category. Modified from Mansingh *et al.*, reference 17.

was shown by extracts of balsam wood and cedar wood, it was considerably less than that shown by farnesyl methyl ether.

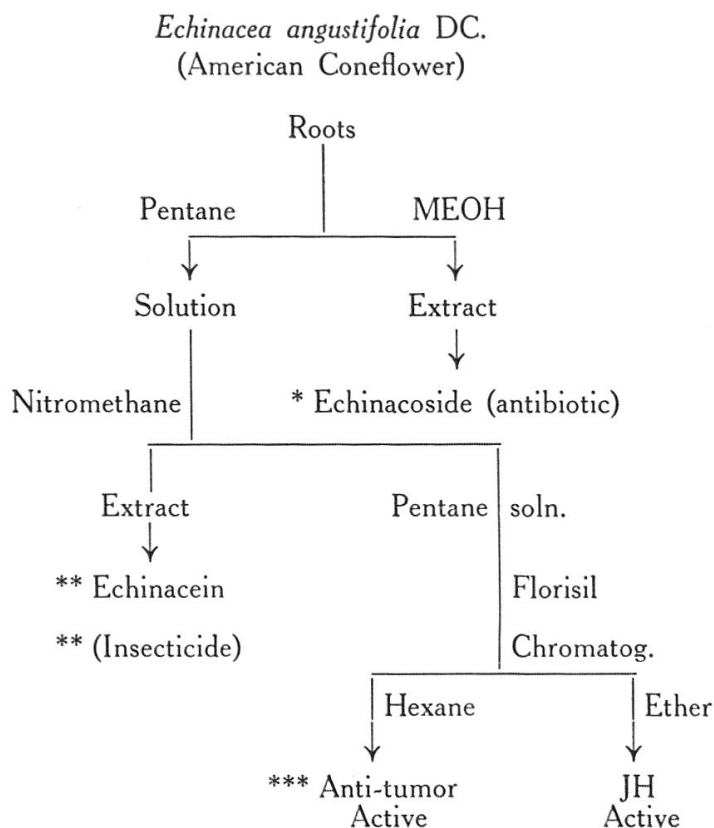
Of the USDA-sponsored research on the JH activity of natural products, one investigation is being carried out by a group in India

under a PL-480 grant administered by the Stored Products Research Branch. This involves the isolation of compounds from feces of the khapra beetle, *Trogoderma granarium*, that cause developmental arrest of larvae of this insect, thus resulting in an effective larval diapause. A preliminary paper on this work has been published in Life Sciences by Karnavar and Nair (18) of the University of Baroda. Lipids extracted from the larval feces with a chloroform-methanol mixture are responsible for the induction of diapause through feeding experiments. The active components are being isolated and their identification attempted.

A group at our Boll Weevil Laboratory in State College, Mississippi (19) is investigating the components of an extract of boll weevil (*Anthonomus grandis*) responsible for inducing complete cessation of growth in young larvae treated topically with chromatographic fractions, as well as pupal-adult intermediates and deformities in treated pupae. Boll weevil larvae treated with the extract moult into a larva-pupa and then emerge to an adultoid with "thalidomide" features. Other treated larvae may show incomplete molt at the tail, a thickened thorax, and retainment of the head cap; pupation is blocked. The effects of topical treatment with a chromatographic fraction of the larval extract on an early boll weevil pupa are formation of an individual with an adult head, but the larval features of the abdomen are retained. Such effects can be induced with amounts less than 0.001 the amount necessary with *Cecropia* JH.

At our laboratories in Beltsville we are attempting to isolate a lipid with JH properties from a methylene chloride extract of adult pink bollworm (*Pectinophora gossypiella*) moths. The extract as well as a chromatographic fraction thereof injected into *Tenebrio* pupae causes eclosion into an adult having severely malformed genitalia.

Finally, also at Beltsville, we have prepared in the past year extracts of some 200 higher plants obtained from various parts of the world and have had them evaluated for JH activity on both *Tenebrio* and *Oncopeltus fasciatus* (milkweed bug). Thus far only one plant has shown considerable promise. The plant is *Echinacea angustifolia*, commonly known as the American coneflower, which grows rather profusely in the states of Kansas, Nebraska, and Missouri. The roots, which until a year ago were available commercially, have been used medicinally in the healing of wounds and inflammations. As can be seen in Figure 1, the roots of *Echinacea* have yielded to us a veritable bonanza of useful chemical compounds. In 1950, Stoll and coworkers (20) isolated and identified a potent antibiotic, echinacoside, from a methanol extract of the roots. Echinacoside is a glucoside of caffeic acid. In 1954 we isolated (21) from the nitromethane-soluble fraction of a pentane extract a highly insecticidal compound, which we designated "echinacein"; we identified it in 1967 as the *N*-isobutylamide of *trans*-2, *cis*-6, *trans*-8, *trans*-10-dodecatetraenoic acid (22). Through Florisil column chromatography of the nitromethane-extracted pentane solution, we have now

Fig. 1. — Fractionation of the Roots of *Echinacea angustifolia*.

\* Stoll *et al.*, reference 20.

\*\* M. Jacobson, references 21 and 22.

\*\*\* D. J. Voaden and M. Jacobson, reference 23.

isolated and identified a hydrocarbon which causes regression of several tumors occurring in humans (23). Finally, by elution of the Florisil column with ether following removal of the oncolytic hydrocarbon, we have obtained a fraction with potent JH activity when applied topically to *Tenebrio* larvae. Our active fraction is as yet only a mixture of triglycerides and diglycerides, which appear to be lending themselves easily to separation. Test results indicate that the pure material, when it is obtained, will be extremely potent.

In conclusion, I wish to thank Mr. R. E. Redfern, U.S.D.A., Beltsville, Maryland, for conducting the bioassays with *Tenebrio* and *Oncopeltus*; Dr. P. A. Hedin, U.S.D.A., State College, Mississippi, for permitting me to discuss his as yet unpublished work; and the Swiss Entomological Society and Swiss Chemical Industry for making it possible for me to attend this Symposium.

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