eluate by thin-layer chromatography indicated that the radioactivity represented H^3-norepinephrine; negligible amounts of deaminated catechol metabolites could be detected. A portion (100 μl) of each chromatographic fraction was placed in scintillation vials containing 1 ml of water and 10 ml of modified Bray's solution (*16*) and counted in a Beckman DPM-100 liquid-scintillation spectrometer. The relative concentrations of H^3-norepinephrine and its metabolites in the control effluent were similar to that found in cat brain tissues after intraventricular injection of this amine (*13*).

The effects of a 30-minute perfusion of *d*-amphetamine (100 μg/ml; total dose of 300 μg) are shown in Fig. 1. There was an immediate and sustained increase in the concentration of H^3-norepinephrine appearing in the effluent when amphetamine was added to the perfusing solution; 30 minutes after the amphetamine perfusion was discontinued, the concentration of H^3-norepinephrine in the effluent was still elevated. Concentrations as low as 6.25 μg/ml (total dose of 18.75 μg) caused a significant increase in the amount of H^3-norepinephrine in the effluent; a maximum response was obtained with 200 μg of *d*-amphetamine per milliliter of perfusing fluid.

After a latent period of 10 to 20 minutes, *d*-amphetamine also increased the concentration of H^3-normetanephrine but not of deaminated-*O*-methyl metabolites appearing in the effluent. The delayed increase of H^3-normetanephrine suggests that H^3-norepinephrine is released (*17*) from brain tissue adjacent to the ventricular system and then partially metabolized by extraneuronal catechol-*O*-methyl transferase (*2*).

After the intraventricular injection of C^{14}-inulin, the addition of *d*-amphetamine to the perfusing fluid did not alter the concentration of this substance in the effluent. Thus, the capacity of *d*-amphetamine to release H^3-norepinephrine from the brain is more specific than that reported in a previous study in which odor stimuli increased the efflux of metabolically inert substances as well as H^3-norepinephrine from the olfactory bulb of rats (*18*).

In contrast to other methods for studying the dynamics of brain catecholamines, which involve chemical or histochemical analysis of these amines and their metabolites at fixed points in time, the cerebral perfusion technique provides an opportunity for continuously monitoring the effects of various stimuli or drugs on the release of amines from the brain. This method has a distinct advantage over the use of push-pull cannulae because the latter significantly damage tissue in the perfused area (*19*). To date, because of limitations imposed by the lack of sensitivity of our analytical methods, we have been unable to detect endogenous catecholamines or their metabolites in the ventricular effluent. Accordingly, it has been necessary to resort to radioactive tracer techniques. Although the regional distribution of intraventricular H^3-norepinephrine is related to that of endogenous catecholamines (*15*), the uptake of H^3-norepinephrine in brain is probably not confined to neurons containing catecholamine so that the norepinephrine which appears in the effluent may not arise exclusively from these structures. This problem may be overcome by labeling brain catecholamines with C^{14}-tyrosine so that C^{14}-catecholamines appearing in the effluent will have been synthesized by and released from noradrenergic or dopaminergic neurons.

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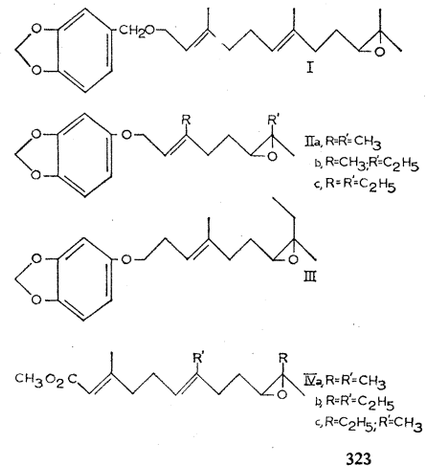
Juvenile Hormone: Activity of Aromatic Terpenoid Ethers

Abstract. *Several aromatic terpenoid ethers possess a high degree of morphogenetic activity when assayed on the yellow mealworm* Tenebrio molitor *L. and the milkweed bug* Oncopeltus fasciatus *(Dallas). The most active compounds were the 3,4-methylenedioxyphenyl ethers of 6,7-epoxygeraniol and the corresponding ethyl-branched homologs.*

Certain commercial insecticide synergists possess significant juvenile hormone activity for several insect species (*1*). The most active synergists were 3,4-methylenedioxybenzyl and -phenyl ethers. In addition, the side chain substituents can be replaced by the sesquiterpenoid alcohol farnesol and its terminal epoxide (I).

In extending these studies I have found that the juvenile hormone (JH) activity of methylenedioxyphenyl terpenoid ethers is significantly greater than that of the corresponding methylenedioxybenzyl analogs and that substitution of 6,7-epoxygeraniol (IIa) for 10,11-epoxyfarnesol as the side chain resulted in a further increase in activity. Various homologs of the geranyl moiety, IIb, IIc, and III, possess an even higher order of JH activity.

Three aromatic terpenoid ethers, V, VI, and VII, were prepared which lack the methylenedioxy moiety. One of



these (VII) displays remarkable species specificity. Most of the new "hybrid" compounds are several hundred times more active on insects than methyl *trans,trans*-10,11-epoxyfarnesenate (IVa) (*2*), and its homologs, the cecropia juvenile hormones (IVb and IVc) (*3, 4*).

The morphogenetic (JH) activity of all compounds was measured in the *Tenebrio* and milkweed bug assays (*1*) by applying the test compounds topically in 1 μl of acetone to the abdomens of newly molted pupae of the yellow mealworm *Tenebrio molitor* (L.) and to last-instar nymphs of the milkweed bug *Oncopeltus fasciatus* (Dallas). Juvenile hormone activity was signaled by the retention of immature characters in the test insects after the final molt toward the adult stage.

Test compounds were synthesized as illustrated in Fig. 1. The specific activity of representative compounds is presented in Table 1. The most active compounds in the *Tenebrio* test were the methylenedioxyphenyl ethers of 6,7-epoxygeraniol (IIa), and its homologs (IIb, IIc, and III). The considerable increase in activity realized with the ethyl-branched analogs is quite surprising, since the ethyl-branched cecropia hormones are but slightly more active (two to three times) than methyl-*trans, trans*-10,11-epoxyfarnesenate (IVa). The slightly greater activity of IIc over IIb and III was minimal, but constant and reproducible. The activities of III and IIb were identical, despite the additional carbon in the chain of III. Compounds IIb, IIc, and III still show significant activity at 0.5 to 0.1 ng.

The importance of the methylenedioxyphenyl group is adequately illustrated by the lesser activity of the derivatives V, VI, and VII; however, the presence of a ketone (VI) or ester (VII) did increase activity significantly. The phenyl derivative (V) required treatment with 10 μg to give a three to four response on *Tenebrio*.

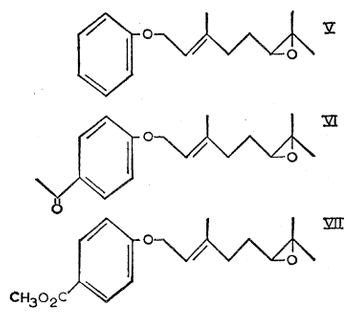


Table 1. Morphogenetic effects of hybrids and related compounds applied topically on the mealworm and milkweed bug. In the "*Tenebrio* genitalia" test, the degrees of modification are represented numerically: 0, no effect; 1, small gin traps present or retention of short urogomphi with genitalia essentially 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; and 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	Responses of test insects to amount (μg) applied					
	Tenebrio			*Oncopeltus*		
	0.001	0.01	0.1	0.001	0.01	0.1
IIa	0	1	3–4	0	0	0–1
IIb	2–3	3	4	0	0–1	2–3
IIc	3	3–4	4	0	0	2–3
III	2–3	3	4	0	0–1	2–3
IVa	0	0	2	0	0	0–1
IVb	0	0	2	0	0	0
V	0	0	0	0	0	1
VI	0	0	2–3	0	0	1
VII	0	0	0–1	0–1	2	3

Although the milkweed bugs were slightly less sensitive to most of these compounds, the structure-activity relations closely paralleled those of *Tenebrio*. Thus, the methylenedioxyphenyl derivatives were the most active, and the presence of ethyl branches definitely increased activity. An exception, however, was the very high activity of VII which inhibited metamorphosis in *Oncopeltus* below 10 ng. This specificity is not unlike that of juvabione (*6*) which has considerable JH activity in another hemipteron, the red linden bug *Pyrrhocoris apterus* (L). Numerous benzoic acid derivatives, patterned after juvabione, have been found to be completely specific for insects in the family Pyrrhocoridae (*7*); however, none of the compounds examined in our study were this specific for any order or family of insects despite the disproportionately high activity of VII for *Oncopeltus*.

The unepoxidized ethers of the aromatic compounds in Table 1 were

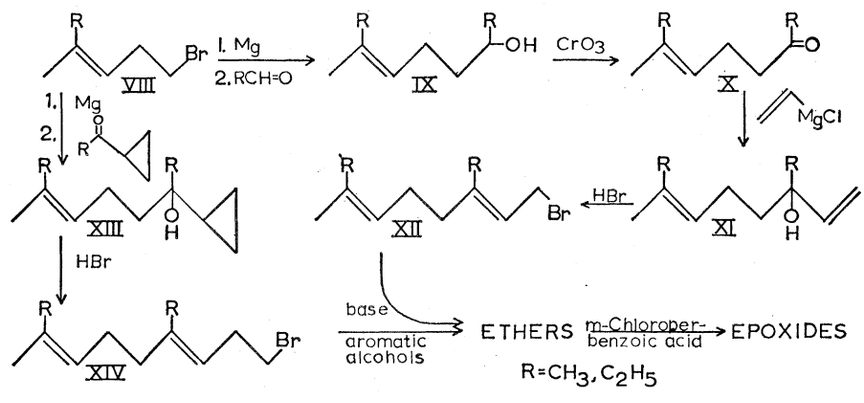


Fig. 1. Grignard reaction of 4-methyl or ethyl-1-bromo-3-pentene (VIII) with the appropriate aldehyde gave IX which was oxidized with chromic acid in acetone to yield the corresponding ketone X. Treatment of X with vinyl magnesium chloride in tetrahydrofuran gave the vinyl alcohol XI which was converted to the *trans* allylic bromide XII in nearly quantitative yield by reaction for 20 minutes with an ice-cold aqueous or acetic acid solution of excess hydrogen bromide. The synthesis of VIII, XIII, and XIV was accomplished by standard methods (*5*). The aromatic terpenoid ethers were prepared by stirring the appropriate bromide with an aromatic alcohol in anhydrous dimethoxyethane with excess powdered potassium hydroxide for 10 to 15 hours. Epoxidation was accomplished with *m*-chloroperbenzoic acid (*2*). All compounds were chromatographed over Florisil and obtained in greater than 99 percent purity, as judged by thin-layer and gas-liquid chromatography. In the case of IIa, V, VI, and VII, advantage was taken of the convenient availability of linalool for the preparation of geranyl bromide. Chemical structures were confirmed by a combination of infrared, nuclear magnetic resonance, and mass spectral data.

approximately one-tenth as active as the terminal epoxides. The methylenedioxybenzyloxy analogs of IIa, IIb, IIc, and III, prepared from piperonyl alcohol by analogous methods, were uniformly one-tenth as active on both test insect species.

The exceptionally high order of activity of these compounds prompted me to examine the effect of their vapors on *Tenebrio*. Thus, 20 freshly molted *Tenebrio* pupae confined in a pint jar, exposed only to the vapors of 0.1 to 0.5 mg of IIa or IIc coated on the bottom quarter of each jar, molted to pupal adult intermediates and second pupae. Thus, the potential utility of the present compounds to control sensitive pest species is compounded by the additional possibility of using them as fumigants.

As candidates for the control of insect pests, these new hybrid compounds would appear to be superior to methyl *trans,trans*-10,11-epoxyfarnesenate and the cecropia hormones by virtue of their greater biological activity and ease of synthesis. Although some of these compounds display biological activity in the picogram range, they are not directly toxic to insects up to a million times the concentration required to prevent metamorphosis. Thus, they do not kill insects in the manner of ordinary insecticides but are effective by deranging development, through interference with metabolic processes that are able to proceed only in the relative absence of JH. Since these compounds are not directly toxic to insects in the conventional sense, imaginative and timely applications to pest species must be developed to fully utilize their practical potential.

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Oyster Ciliary Inhibition by Cystic Fibrosis Factor

Abstract. *Ciliary inhibition in oysters serves as an assay in identifying a serum factor in cystic fibrosis patients and heterozygotes. Serums from 47 patients with cystic fibrosis and 19 heterozygotes caused ciliary cessation within 35 minutes, whereas serums from only 2 of 64 individuals without cystic fibrosis inhibited ciliary activity within this time.*

Serums from patients with cystic fibrosis (CF) inhibit ciliary synchrony of rabbit tracheal explants incubated for 4 to 6 days in culture (*1*). Concentrated preparations of serums from known CF heterozygotes also cause cilia rhythm to become asynchronous. The serum factor responsible was described by Spock *et al.* (*1*) to be heat-labile and nondialyzable. Additional evidence of an abnormal factor in cystic fibrosis was found by Mangos *et al.* (*2*), who described an inhibitor of sodium transport present in sweat and saliva from CF patients; this may or may not be the same as the serum abnormality.

We have tested the effect of serum from CF patients on the ciliary activity of oyster gills, a readily available source which eliminates lengthy incubation. Gill tissue from fresh oysters (*Crassostrea virginica*) was removed, and vertical sections (3 by 3 mm) were suspended in filtered seawater in a hanging drop preparation (*3*). After cilia were observed to be active in seawater (under phase-contrast microscopy), the seawater was replaced by serum, and the preparation was tightly sealed with wax and examined at various times. The time in which the cilia stopped beating was recorded for each serum. Sealed cilia preparations in seawater remained active for approximately 1 hour. The typical reaction produced by serum from CF patients on oyster tissue was an immediate expulsion of debris from tubules between the gill mounds followed by cessation of the mound cilia. In Fig. 1, the cilia in normal serum are compared to gill cilia exposed for 15 minutes to serum from CF patients. Typical accumulation of debris covers the cilia and mounds in the serum preparation from CF patients.

The time required for ciliary cessation was approximately the same on duplicate runs of the same individual's serum; however, the age and condition of the serum affected the obtaining of repeatable results. After serum had been frozen and thawed a number of times, the capacity to stop ciliary action was lost. Hemolyzed serum failed to give repeatable results. Because of the variation in ciliary activity of oyster gills, it was necessary to use controls with each series of unknown serums, including seawater, serum from normal individuals, and serum from CF patients. Unknowns were examined every 5 minutes and tested in duplicate on gill cilia from different oysters.

In a study in which the identity of all samples was unknown to the examiners, the effects of serums from 47 CF patients, 19 CF heterozygotes, 25 allergic children, and 39 healthy individuals were observed (*4*). Serums from 62 individuals without CF did not inhibit ciliary action, which persisted for 40 to 50 minutes (Fig. 2). The serums from children with allergic rhinitis and bronchial asthma failed to inhibit the oyster cilia; movement persisted from 40 to 50 minutes. Serum from normal individuals did not overlap CF homozy-

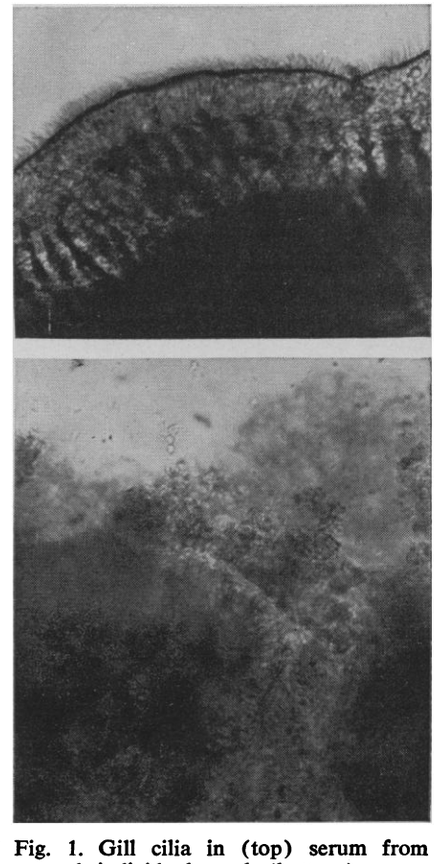

Fig. 1. Gill cilia in (top) serum from normal individuals and (bottom) serum from CF patients. Typical accumulation of debris covers the cilia and mounds in the serum preparation from CF patients (phase contrast, × 160).