

shows that one of the two rings was cleaved. Moreover, further degradation of this metabolite occurred; thus, when washed cell suspensions were incubated with *p*-chlorophenylacetic acid, a yellow-colored product accumulated. This product had an absorption maximum of 379 nm in neutral to basic solutions which disappeared upon acidification. Nevertheless, no chloride was released microbiologically.

Furthermore, when a suspension of the cells was incubated with 1,1-diphenyl-2,2,2-trichloroethane, a product appeared that was identified by its melting point, infrared spectrum, and by mass spectral analyses as 2-phenyl-3,3,3-trichloropropionic acid. The infrared spectrum showed bands at 1700 and 690  $\text{cm}^{-1}$  characteristic of an aromatic acid. Mass spectrometry showed (i) a mass of 252; (ii) the loss of HCl, carboxyl, and chlorine from the parent ion; (iii) a base peak at mass-to-charge ratio (*m/e*) 102, representing a loss of three chlorines and a carboxyl; and (iv) a peak at *m/e* 77 for the phenyl ion. One of the two rings in this substrate too, therefore, was opened.

It has been reported that DDT may be converted by *Culex tritaeniorhynchus* larvae to *p*-chlorobenzoic acid (6). Although no such intermediate has been observed to be excreted by the bacterium, it has not been excluded either. Nevertheless, the evidence for a role for *p*-chlorobenzoic acid in DDT degradation must be considered as equivocal in view of the lack of a rigorous identification of the compound.

The present findings that diphenylmethane, *p,p'*-dichlorodiphenylmethane, and 1,1-diphenyl-2,2,2-trichloroethane are attacked, that at least one and sometimes both of the benzene rings are cleaved, and that there is extensive microbial modification of the chemicals focus attention on the ultimate fate of DDT in soil and water, environments that have received enormous quantities of this insecticide. Microorganisms under anaerobic conditions are capable of converting the parent pesticidal molecule to compounds of the kind metabolized aerobically by *Hydrogenomonas* sp. (1), so that a biological model now exists for tracing the pathway of DDT decomposition in nature.

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## Peptides with Juvenile Hormone Activity

**Abstract.** Peptide derivatives of juvenile hormone analogs which show substantial hormonal activity for certain insects were prepared. The most active compound, L-isoleucyl-L-alanyl-*p*-aminobenzoic acid ethyl ester, was up to twice as active as juvabione. Like juvabione, the peptide analog showed selective action on pyrrhocorid bugs.

The juvenile hormone (JH) analogs are mainly aliphatic or monocyclic sesquiterpenes or aliphatic monoterpenes attached to para-substituted aromatic rings. A few examples of active compounds without isoprenoid structure include dodecylmethylether (1) and insecticide synergists of the sesoxane type (2). We were interested in polypeptide chains that bear a general resemblance to polyisoprenoids and have prepared and assayed several peptides which mimic selected terpenic and terpenoid models.

The first compound investigated was the methyl ester of 3,7,11-trimethyl-2-dodecenic acid (compound 1), which is active on both hemipteran larvae and pupae of the beetle *Tenebrio* (Table 1). Its partly peptidic counterpart (2) was prepared from amino acids L-valine and L-alanine attached to a short-chained, unsaturated, dicarboxylic acid ester. One end of the molecule contains the necessary  $-\text{CH}_2\cdot\text{C}(\text{CH}_3)\cdot\text{CH}\cdot\text{COOR}$

structure, which in many straight chain terpenic analogs is necessary for JH activity (3). In addition, compound 2 contains a carbomethoxy group on the

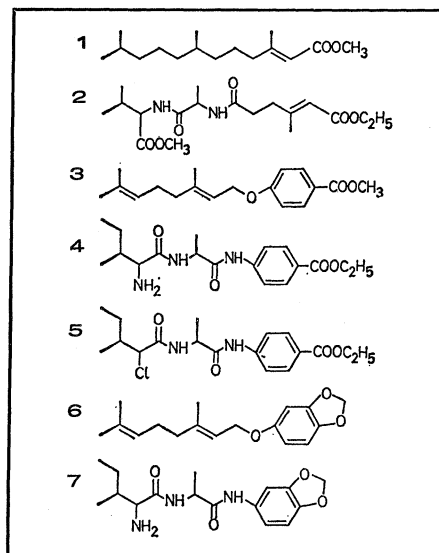


Table 1. Juvenile hormone activity. The values indicate the amount of the substances (micrograms per specimen) which caused formation of half-larval (hemipterans) or half-pupal (*Tenebrio*) adultoids. For topical applications we used standard 1- $\mu\text{l}$  drops of acetone solution and for injections 1  $\mu\text{l}$  of olive oil; freshly molted last instar larvae or freshly molted pupae (0 to 20 hours) were used for the assays.

Compound	Pyrrhocoridae		Pentatomidae <i>Graphosoma italicum</i> Topical (larvae)	Tenebrionidae <i>Tenebrio molitor</i>	
	<i>Pyrrhocoris apterus</i> Topical (larvae)	<i>Dysdercus cingulatus</i> Topical (larvae)		Injections (pupae)	Topical (pupae)
1	5	10	100	1	10
2	> 1000	> 1000	> 1000	> 1000	> 1000
3	0.5	0.5	0.8	10	100
4	0.5	0.5	> 1000	> 1000	> 1000
5	0.5	0.2	> 1000	> 1000	> 1000
6	5	5	1		0.3
7	100	100	> 1000	500	
$\pm$ Juvabione	1	0.5	> 1000	> 1000	> 1000
Methyl farnesoate	50	50	50	10	

other end of the molecule. The bioassays (Table 1) revealed that compound 2 had no juvenile hormone activity even when tested in high concentrations.

We next investigated JH analogs containing an aromatic ring. As a model compound we selected the geranyl ether of *p*-hydroxybenzoic acid (Table 1, compound 3) (4). As the peptidic counterpart, we prepared the ethyl ester of L-isoleucyl-L-alanyl-*p*-aminobenzoic acid (compound 4). Though this substance shows an overall similarity with 3, especially with respect to the general size of the molecule and the locations of the alkyl substituents, some of the methylene groups of compound 3 are replaced by =CO and —NH— groups. Isoprenoid juvenile hormone analogs are already known in which methylene groups are replaced by both =CO (5) and —NH— (6) groups.

In contrast with 2, compound 4 exhibits substantial juvenile hormone activity for pyrrhocorid bugs when dissolved in olive oil and injected or when dissolved in acetone and topically applied on the body surface of freshly molted final (5th) instar larvae. Doses of 0.5 µg in topical applications cause the appearance of half-larval adultoids. This activity for the Pyrrhocoridae is similar to that found for the model terpenoid compound 3. It is somewhat more active than juvabione and much more active than methyl farnesoate, farnesol, and many other terpenic JH analogs. Like the juvabione-type compounds, the peptidic ester 4 shows a high degree of specificity and appears to be inactive for the pentatomid bugs or for the Coleopteran *Tenebrio*. As far as we know it is the first peptide with JH activity.

The JH activity of some terpenic compounds can be enhanced by introduction of one or more chlorine atoms (7). We therefore exchanged the amino group of isoleucine in 4 for the chlorine atom (by chlorinating deamination) (8) to obtain the ethyl ester of 2-chloro-3-methyl-valeryl-L-alanyl-*p*-aminobenzoic acid (compound 5). This substance showed approximately the same activity as the original tripeptide ester 4. Consequently, no potentiation of the hormonal activity was realized by the introduction of the chloride atom.

Replacement of the ester group in aromatic juvenile hormone analogs by

the methylenedioxy group often causes a shift in species specificity. Such compounds are generally more active on pupae of Coleoptera and less active on Hemiptera. As an example of this type of compound we selected the 3,4-methylenedioxyphenylether of geraniol (6) (4). We prepared an analogous compound to our tripeptide ester 4 with 3,4-methylenedioxyaniline instead of the ester of *p*-aminobenzoic acid. This isoleucyl-alanyl-methylenedioxyaniline (7) showed decreased activity for the hemipterans and low but definite juvenile activity for *Tenebrio* pupae.

Our results document a pharmacobiological similarity between determined isoprenoid and peptide compounds with respect to JH activity. The data suggest that the relations between structure and activity may in principle be similar in both peptidic and isoprenoid JH analogs.

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8. The substance 2 was obtained as a crystalline solid [melting point 121 to 122°C;  $[\alpha]_D^{25}$ , 45.5° (2 percent in methanol)] by means of dicyclohexylcarbodiimide condensation of 5-ethoxycarbonyl-4-methyl-4-pentenoic acid (mixture of *cis-trans* isomers) with methyl ester of L-valyl-L-alanine. Compound 4 was obtained in crystalline form [melting point, 161 to 162°C;  $[\alpha]_D^{25}$ , 69.2° (2 percent in methanol)]. The tripeptide ester 4 and the dipeptide derivative 7 were prepared by a stepwise approach starting from *p*-aminobenzoic acid ethyl ester or from 3,4-methylenedioxyaniline, respectively. The compound 4 was synthesized from benzyloxycarbonylamino acids as intermediates. The protecting group was removed by treatment with HBr and acetic acid. Nitrophenylsulphenylamino acids were used for the synthesis of 7, and the protecting group was removed by treatment with HCl and methanol. Dicyclohexylcarbodiimide served as condensing agent in both cases. Compound 7 formed an oily material (mass spectrum peaks at 321,290,262,238,208,182,157,137,100,88, and 44). The chloroderivative 5, obtained by the action of NaNO<sub>2</sub> in HCl, was noncrystalline material (mass spectrum peaks at 368,350,332,323,287,263,221,204,192,176,168,165,120, and 97). All the crystalline compounds gave correct elemental analysis data.

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## Mueller-Lyer Illusion: Effect of Age, Lightness Contrast, and Hue

**Abstract.** *Mueller-Lyer figures produced by lightness contrast (white on black) and by hue contrast in the absence of lightness contrast (red, yellow, green, or blue on gray) were presented to subjects aged nine to adult. Contrary to Piagetian expectations, the illusion magnitudes resulting from the colored figures did not decline with age.*

Piaget (1) has argued that the well-known diminution of the Mueller-Lyer illusion with age (2) and with repeated trials (3) is due to an increase in perceptual activity which involves deceleration, or comparative looks at various parts of the figure. I proposed (2) that the decline in the magnitude of the illusion, at least under conditions of fixation using a small figure exposed tachistoscopically, is due to the decline in sensitivity to contours which are produced by brightness or lightness contrast (2).

The research reported here shows that there is no decline in magnitude of the illusion as a function of age when the Mueller-Lyer figure is produced by hue contrast. The Piagetian explanation, therefore, is not a necessary one, al-

though it may be sufficient under certain conditions.

Piaget says, "If our schema is correct, an optico-geometric illusion has to do with unequal densities of encounters on the elements of the figure, and thereby with their incomplete couplings. It follows that compensations which lead to a diminution of errors must consist in an equalisation of the density of encounters and in the completion of couplings: and it is precisely in such a situation that we may expect to find repeated explorations and improvements with practice" (1, p. 147). He also says, "... the absolute value (of the illusion) itself depends on the relative completeness of the couplings, so it is understandable that these 'primary' errors diminish with age.