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111. Biosynthesis of Juvenile Hormone in the *Cecropia* Moth. Labelling Pattern from 1-[^{14}C]-Propionate through Degradation to Single Carbon Atom Derivatives*

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(13. II. 75)

Summary. In the adult male *cecropia* moth, 1-[^{14}C]-propionate is incorporated specifically into juvenile hormone I (**1**). By chemical degradation it was found that only C(7) and C(11) are labelled, each carbon atom bearing 50% of the radioactivity originally present in *JH-I*. It is concluded that propionate serves as a precursor of homomevalonate, which in turn is a precursor for *JH-I*. Application of 2-[^{14}C]-propionate and 3-[^{14}C]-propionate leads to extensive randomization of the label. Apparently propionate is metabolized such that C(2) and C(3) can be reused as smaller fragments–probably acetate–while C(1) is either highly diluted or removed from the propionate in a metabolically inactive form.

The insect juvenile hormones I and II (*JH-I*, **1**; *JH-II*, **2**) are the only known terpenoid compounds where methyl groups biosynthetically derived from C(3') of mevalonate are formally replaced by ethyl groups. They were discovered in adult males of the saturniid moth *Hyalophora cecropia* [1] [2] and were also isolated from the related species *Hyalophora gloveri* [3] and *Samia cynthia* [4]. Juvenile hormone is produced by *corpora allata*, a pair of glands associated with the insect brain. It was

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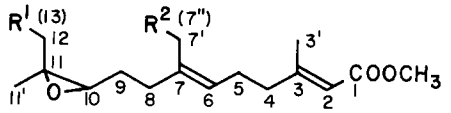
possible to maintain *corpora allata* of *H. cecropia in vitro* and to extract *JH-I* from the incubation medium [5]. The *corpora allata* of the sphingid moth *Manduca sexta* under similar conditions produced no *JH-I* (**1**), but the lower homologues *JH-II* (**2**) and *JH-III* (**3**) [6]. The only other known biological source of either *JH-I* or *JH-II* is the explanted and cultured *corpora allata* of the noctiid moth *Heliothis virescens* [7]. *JH-III* has recently been isolated from whole insect extracts or *corpora allata in vitro* of several species from the orders Orthoptera, Coleoptera, and Hymenoptera [8].



1 $R^1 = R^2 = CH_3$

2 $R^1 = CH_3$, $R^2 = H$

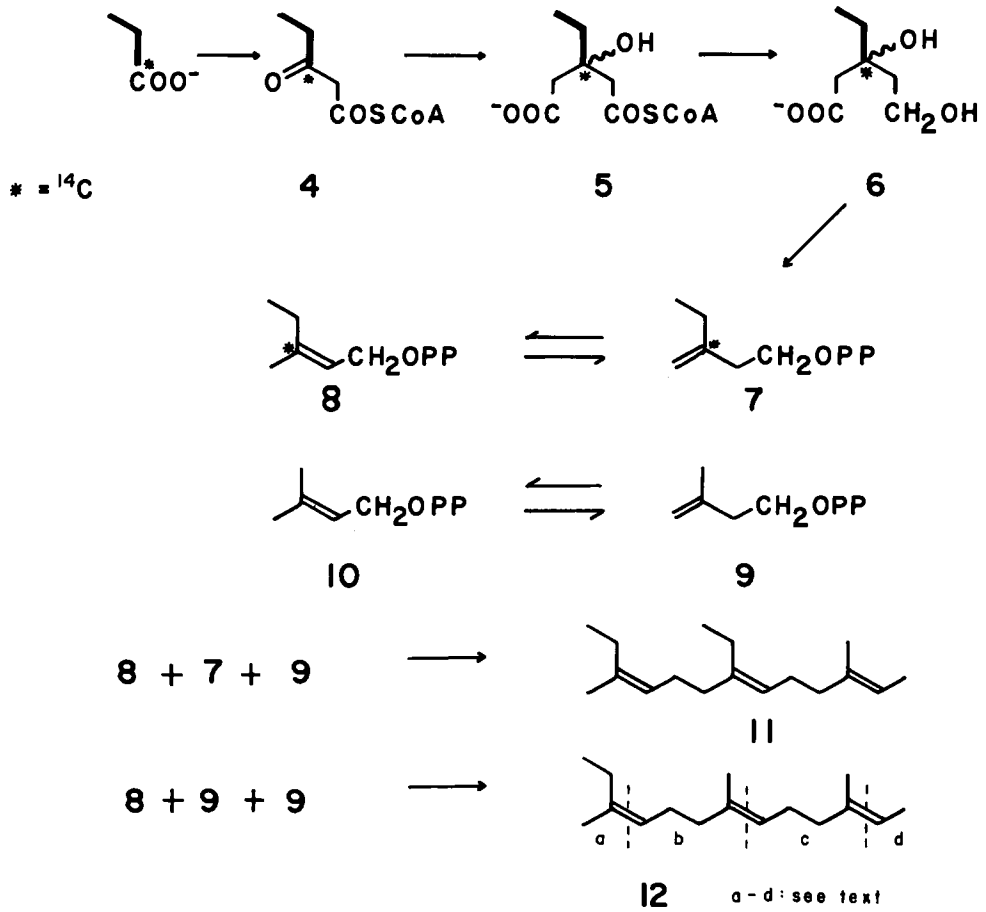
3 $R^1 = R^2 = H$

The structure of *JH-I* is that of an acyclic sesquiterpene with the additional methyl groups C(7") and C(13). Therefore, it could be assumed that its biosynthesis largely corresponds to that of farnesol. The two ethyl groups at C(7) and C(11) could be produced by addition of C_1-units to an intermediate precursor. Biosynthetic homologizations of terpenoids are known in the phytosterol series where an S-methyl group of methionine is transferred to a double bond in the side chain. A second methyl group may be added, completing the synthesis of a C_2-branch at C(24) of the steroid [9]. Attempts to use ([^{14}C]-methyl)-methionine as a donor of methyl groups for *JH-I* and *JH-II* were only partly successful: in *H. cecropia in vivo*, the label was not incorporated in the carbon skeleton of the hormones but exclusively in the ester methoxy group [10]. The application of this labelling method in organ cultures led to discovery of the *JH-II* and *JH-III* production by *corpora allata* of *Manduca sexta in vitro* [6]. Again, the label from ([^{14}C]-methyl)-methionine was recovered only in the methoxy group of the hormone.

As an alternative to the methylation of a terpenoid intermediate, *JH-I* and *JH-II* could also be biosynthesized from propionate and acetate. The biosynthesis (*Scheme 1*) of the homosesquiterpenes *JH-I* and *JH-II* would have to commence with the production of propionyl-acetate (**4**) in its metabolically active form. Condensation with another acetate leads by way of 3-ethyl-3-hydroxy-glutarate (**5**) to homomevalonate (**6**). **6** would be converted to 3-ethyl-but-3-en-ylpyrophosphate (**7**), which can be isomerized to 3-methyl-pent-2-en-ylpyrophosphate (**8**). The biosynthesis of all three juvenile hormones requires the presence of the mevalonate metabolite **9**. For *JH-III*, dimethylallyl pyrophosphate (**10**) is necessary in addition. The carbon skeleton **11** of *JH-I* is derived by condensation of **8**, **7**, and **9** while that of *JH-II* (**12**) is formed from **8** and **9** only. *JH-III* finally is produced from **10** and **9**.

First attempts to verify the validity of the biosynthesis scheme (*Scheme 1*) in *H. cecropia in vivo* led to inconclusive results [10a]: Acetate served as precursor for both hormones, but mevalonate as the assumed source of the carbon atoms 1,2,3,4 and 3' was not incorporated into *JH-I* or *JH-II*. In the initial experiments, 1-[^{14}C]-

Scheme 1

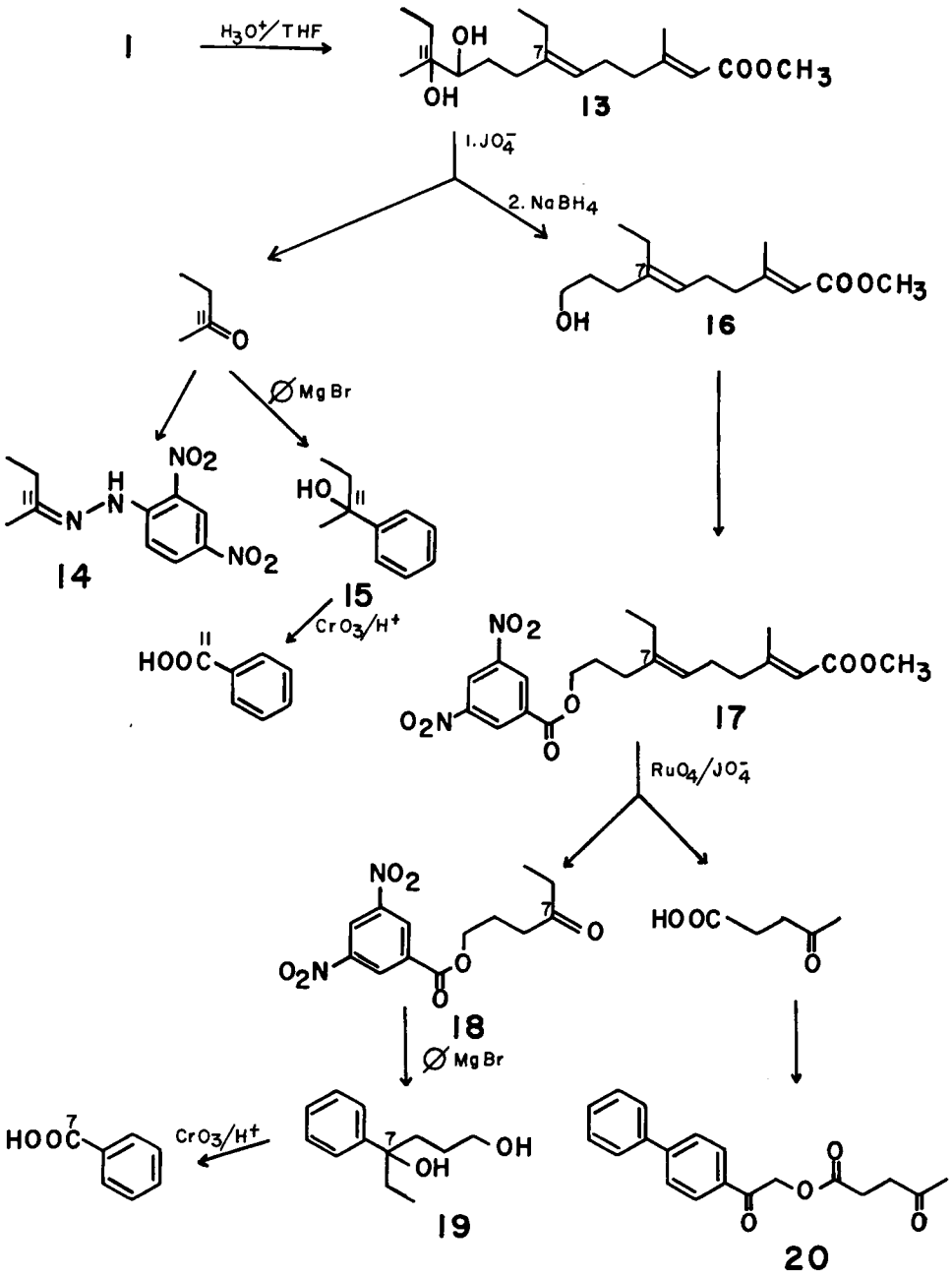


propionate (2.5 μCi/moth) was not incorporated significantly in *JH-I* or *JH-II*. After administration of larger doses (50 μCi/moth), however, strong evidence was collected that 1-[^{14}C]-propionate is incorporated into both hormones as an intact C_3-unit [11]. In contrast to *H. cecropia in vivo, corpora allata* of *Manduca sexta in vitro* utilize not only acetate and propionate but also mevalonate for synthesis of the hormones [12]. The labelling pattern in *JH-II* after incorporation of the three precursors was partly determined by cleavage of the molecule into four fragments (*a, b, c, d* in formula **12**, *Scheme 1*). An analogous degradation was applied to *JH-III* (**3**). The results were consistent with the postulated biosynthetic pathway for the formation of *JH-II* and *JH-III* as outlined in *Scheme 1*.

We now have applied the degradation sequence described by *Schooley et al.* [12] to *JH-I* which has been isolated from adult male *H. cecropia* after injection of 1-[^{14}C]-propionate. In addition, further reactions were performed which allowed the determination of label present in the single carbon atoms C(7) and C(11).

Degradation of *JH-I* (*cf.* [12]). – After dilution with unlabelled *JH-I*, the preparations were submitted to the degradation sequence outlined in *Scheme 2*. Acid catalyzed hydration of the oxirane ring converted **1** to the ester-diol **13**, which was

Scheme 2



cleaved with sodium periodate in aqueous methanol to a C_{14}-aldehyde and butanone. The butanone was distilled into a solution of 2,4-dinitrophenylhydrazine in ethanol/phosphoric acid to form the dinitrophenylhydrazone (butanone-DNPH, **14**). Alternatively, after dilution with unlabelled butanone, it was distilled into an ethereal solution of phenylmagnesium bromide. The resulting 2-phenyl-butan-2-ol (**15**) was oxidized with chromic/sulfuric acid to a benzoic acid containing C(11) of *JH-I*.

The C_{14}-aldehyde of the periodate cleavage was treated with sodium borohydride and isolated as the hydroxy-ester **16**. Reaction with 3,5-dinitrobenzoyl chloride (DNB-chloride) converted **16** to the DNB-ester **17** which was cleaved with ruthenium tetroxide/sodium periodate/periodic acid to the DNB-ketone **18** and levulinic acid. The levulinic acid was isolated as the *p*-phenyl-phenacyl ester **20**. Reaction of **18** with phenylmagnesium bromide produced the phenyl-hexanediol **19** which in turn was oxidized with chromic/sulfuric acid to a benzoic acid containing C(7) of *JH-I*.

The radioactivity of the products was measured by liquid scintillation counting. The yields of the reactions and the specific activity of the products were calculated either through use of 7-([^{3}H]-ethyl)-*JH-I* as an internal standard (see below) or from UV.-spectra. For this purpose, radioinactive reference compounds were prepared (see experimental part) and their UV.-absorption determined. The following molar absorptivities were found: *JH-III* (**3**): $\varepsilon_{220} = 13{,}800$ (methanol) (*cf.* [8d]) (the same value was used for *JH-I* (**1**) and for the esterdiol **13**); *butanone 2,4-dinitrophenylhydrazone* (**14**): $\varepsilon_{360} = 21{,}900$ (ethanol); *2-phenyl-butan-2-ol* (**15**): $\varepsilon_{257} = 215$ (ether) (*cf.* [13]); 7'-nor-**17**: $\varepsilon_{228} = 28{,}100$; $\varepsilon_{300} = 396$ (cyclohexane) (the same value was used for the *JH-I* derivative **17**); *DNB-ketone* **18**: $\varepsilon_{222} = 24{,}200$; $\varepsilon_{300} = 415$ (cyclohexane); *4-phenyl-1,4-hexanediol* (**19**): $\varepsilon_{257.5} = 203$ (methanol); p-*phenyl-phenacyl levulinate* **20**: $\varepsilon_{285} = 24{,}600$ (methanol); *benzoic acid*: $\varepsilon_{272} = 854$; $\varepsilon_{279} = 691$ (methanol) [*cf.* [14]).

Results and Discussion. – Three series of degradations (Table 1) were performed. The starting material for the first and the second were the combined *JH-I* fractions of all incorporation experiments with 1-[^{14}C]-propionate described elsewhere [11]. This sample was divided into two batches which were diluted individually with radioinactive *JH-I*. 7-([^{3}H]-ethyl)-*JH-I* was added in order to facilitate and control the determination of the specific activities of all derivatives containing the 7-ethyl-group of *JH-I*[1]). For the first degradation, 2.26 mg *JH-I* was used containing 28,600 dpm ^{3}H and 19,000 dpm ^{14}C. The second degradation was performed on 1.56 mg *JH-I* with 14,000 dpm ^{3}H and 7,350 dpm ^{14}C. The amount of starting material was calculated from the specific ^{3}H-activity of the DNB-ester **17**, which in turn was determined by UV.-spectrometry.

In the first degradation series, the C_4-fragment, representing C(11), C(11'), C(12), and C(13) of *JH-I*, was isolated as butanone 2,4-dinitrophenylhydrazone (**14**). It contained one half of the original [^{14}C]-activity. In very good agreement the C_{14}-

[1]) The quality of the commercial standard in turn was checked by comparison with ([^{14}C]-methoxy)-*JH-I* which had been produced by transesterification of *JH-I* with [^{14}C]-methanol. Degradation of a mixture of these two reference compounds containing 143,000 dpm ^{3}H/μmol and 30,000 dpm ^{14}C/μmol gave the ester-diol **13** with 155,000 dpm ^{3}H/μmol and 34,500 dpm ^{14}C/μmol and the dinitrobenzoate **17** with 137,000 dpm ^{3}H/μmol and 29,800 dpm ^{14}C/μmol.

Table 1. *Degradation of* JH-I *after Incorporation of 1-[^{14}C]-Propionate*

	First Degradation			Second Degradation			Third Degradation	
	^{14}C dpm/μmol	$^{3}H/^{14}C$ [a]	$\%^{14}C$ [b]	^{14}C dpm/μmol	$^{3}H/^{14}C$ [a]	$\%^{14}C$ [b]	^{14}C dpm/μmol	$\%^{14}C$ [b]
JH **1**	2470	1.50 ± 0.05	100	1380	1.91 ± 0.10	100	6160 ± 300	89
ester-diol **13**	2420	1.54 ± 0.06	98	1240	2.13 ± 0.11	90	6890 ± 350	100
butanone-DNPH **14**	1270		52					
phenyl-butanol **15**				56.7		100 [c]	97.7 ± 4.9	[g] 100
benzoic acid from **15**				61.0		108	93.6 ± 4.7	96
hydroxy-ester **16**	1270	2.92 ± 0.12	51	632	4.18 ± 0.21	46 [d]		
DNB-ester **17**	1280	2.91 ± 0.12	52	704	3.76 ± 0.19	51	3520 ± 180	[h] 51
DNB-ester **17**				1030	3.16 ± 0.16	100 [e]		
DNB-ketone **18**				1010	3.20 ± 0.16	98	3920 ± 200	57
phenyl-hexanediol **19**				27.2	3.32 ± 0.17	100 [f]	256 ± 12	[i] 100
benzoic acid from **19**				26.9		99	257 ± 12	100
phenacyl levulinate **20**							nil	

[a] 7-([^{3}H]-Ethyl)-*JH-I* was added as an internal standard;
[b] specific activity of derivative relative to specific activity (100%) of the starting material in each degradation sequence;
[c] after periodate cleavage of **13**, 4.0 μl unlabelled butanone was added to the reaction mixture;
[d] 46% relative to **1**;
[e] **17** of the first degradation combined with **17** of the second;
[f] before the reaction with phenylmagnesium bromide, **18** had been diluted to a specific activity of 29.5 dpm ^{14}C/μmol;
[g] after periodate cleavage of **13**, 10 μl of unlabelled butanone had been added to the reaction mixture;
[h] 51% relative to **13**;
[i] before the reaction with phenylmagnesium bromide, **18** had been diluted to 244 dpm ^{14}C/μmol.

fragment, isolated as the hydroxy-ester **16** and its dinitrobenzoate **17**, accounted for the other half of the specific [^{14}C]-activity as indicated by the increase of the $^{3}H/^{14}C$ ratio by a factor of about 2. During the second degradation, the C_4-fragment butanone was diluted with unlabelled material and converted to 2-phenyl-butan-2-ol (**15**), which subsequently was oxidized to benzoic acid. The benzoic acid and **15** had the same specific ^{14}C-activity. This result, together with the result of the first degradation, demonstrates the presence of 50% of the [^{14}C]-activity of *JH-I* in C(11).

In the second degradation sequence, the hydroxy-ester **16** and its derivative **17** again contained 50% of the [^{14}C]-label. The dinitrobenzoates **17** of both degradation series were combined to give a new starting material with a $^{3}H/^{14}C$ ratio of 3.16. After cleavage with ruthenium tetroxide/periodate, the radioactivity was completely retained in the 4-oxo-hexanyl dinitrobenzoate **18**. Its derivative **19** showed within the limits of error the same $^{3}H/^{14}C$ ratio. Oxidation of the phenylhexanediol **19** with chromic/sulfuric acid led to a benzoic acid which had the same specific ^{14}C-activity as **19**. Consequently, one half of the specific ^{14}C-activity of *JH-I* was contained in C(7).

For the third degradation, a fresh batch of ^{14}C-labelled *JH-I* was prepared by injection of 1.0 mCi (6.7 mg) 1-[^{14}C]-propionate in ten 1–2 day old male moths. After an incubation period of 28–48 hours, *JH-I* was isolated. It contained 60,000 dpm ^{14}C corresponding to an incorporation ratio of 0.0027%. 26,400 dpm of this preparation were diluted with radioinactive *JH-I* to 1.26 mg. No [^{3}H]-labelled *JH-I* was added in this experiment.

The results, based on calculation of the specific activities from UV.-data only, agree completely with those of the other degradation series. In this experiment the levulinic acid was isolated as the *p*-phenyl-phenacyl ester (**20**) which, as expected, did not contain [^{14}C]-activity.

In contrast to 1-[^{14}C]-propionate, the label of 2-[^{14}C]- and 3-[^{14}C]-propionate is not incorporated exclusively at the positions indicated by the biosynthesis scheme *Scheme 1*. *JH-I* from labelling experiments with the latter two compounds [11] was cleaved to the hydroxy-ester **16** (Table 2). In both experiments 7-([^{3}H]-ethyl)-*JH-I* was added as an internal standard. If the propionate had been incorporated specifically, the ^{3}H/^{14}C ratio was expected to increase by a factor of 2. For complete randomization of the ^{14}C-label over 17 C-atoms a ^{3}H/^{14}C ratio of 3.56 in **16** or **17** was

Table 2. *Degradation of* JH-I *after incorporation of 2-[^{14}C]- and 3-[^{14}C]-propionate.* 7-([^{3}H]-ethyl)-*JH-I has been added as an internal standard*)

Derivative	2-[^{14}C]-propionate ^{3}H/^{14}C	3-[^{14}C]-propionate ^{3}H/^{14}C
JH-I **1**	2.71 ± 0.14	2.75 ± 0.14
ester-diol **13**	2.72 ± 0.14	2.82 ± 0.14
hydroxyester **16**	3.31 ± 0.16	4.10 ± 0.20
DNB-ester **17**	3.59 ± 0.18	

calculated. Comparison of these values with the experimental data indicate complete randomization of the 2-[^{14}C]-propionate label and about 70% randomization during incorporation of 3-[^{14}C]-propionate. Partial randomization of the label of 2-[^{14}C]-propionate had also been observed during the study of *JH-II* biosynthesis *in vitro* [12]. Our results can be interpreted thus, that propionate is metabolized *in vivo* to acetate containing C(2) and C(3) which is reused in biosynthetic sequences. The C(1) fragment is either eliminated or highly diluted. Consequently, ^{14}C at C(1) of propionate is incorporated in *JH-I* only as propionate while ^{14}C at C(2) and C(3) can also be incorporated *via* acetate.

Our studies on the biosynthesis of *JH-I* show unequivocally that the ethyl groups at C(7) and C(11) are formed by incorporation of propionate into the molecule. Since *corpora allata* of *M. sexta in vitro* can utilize mevalonate [12] and homomevalonate for the biosynthesis of *JH-I* (see below) [15] and *JH-II* [16] there is no reasonable doubt that the biosynthesis of the juvenile hormones otherwise follow the established route of terpene biosynthesis.

In another laboratory *JH-I* has been obtained from *corpora allata* of *Heliothis virescens in vitro*. After labelling through incorporation of 1-[^{14}C]-propionate, the hormone was cleaved into four fragments analogous to the degradation of *JH-II* as

indicated by **12** (*Scheme 1*). The distribution of the label in this preparation was found to be in complete agreement with the established biosynthesis scheme [7].

An interesting object for further studies will be the specificity of the prenyl transferases catalyzing the linkage **8** to **7** and (**8** + **7**) to **9** (*Scheme 1*). In *H. cecropia in vivo*, **7** can be replaced by **9** in the first step of the prenyl transferase-reaction, as indicated by the presence of 10–30% *JH-II*. Replacement of **8** by **10** in the first step or of **9** by **7** in the second step of the prenyl transferase-reaction seems to be very unlikely. In spite of some effort, we never found *JH-III* or the 3-ethyl homologue of *JH-I* (*JH*-0). Our studies on *in vitro* systems indicate [15] that fresh *corpora allata* of female *M. sexta* produce approximately equal amounts of *JH-II* and *JH-III*. When homomevalonate is added to the medium, the *JH-II* production is stimulated about 5 fold. The *JH-III* production remains at the previous level and *JH-I* in amounts equal to *JH-III* can be isolated. *Corpora allata* of *Periplaneta americana* under the same culture conditions produce only *JH-III* and cannot be induced by homomevalonate to synthesize any of the other hormones. It remains to be seen whether this specificity of juvenile hormone biosynthesis depends upon the integrity of the whole gland or whether it reflects a specificity of the prenyl transferase-reaction.

We wish to thank Dr. *Jennings* for prepublication information about his results concerning the juvenile hormone of *H. virescens*. This work was supported by a grant from the *National Science Foundation* (GB-32334X).

Experimental Part

General remarks. – M.p. were measured on a melting point apparatus according to Dr. *Tottoli* (Glasapparatefabrik *Büchi*, Flawil, Switzerland); they are uncorrected. UV./VIS.-spectra were measured on a *Zeiss* Model PMQ II spectrophotometer. NMR.-spectra (60 MHz) were obtained on a *Varian* T-60 NMR-spectrometer with TMS ($\delta = 0$ ppm) as internal standard. Mass spectra were recorded with an *LKB* 9000 gas chromatograph/mass spectrometer: 300 cm glass column with 3% SE-30 on Gas Chrom Q, separator 240°, ion source 270°, electron energy 70 eV.

Chromatography. – Thin layer chromatography (TLC.), if not otherwise indicated, was performed on glass plates coated with 0.25 or 0.5 mm silicagel HF_{254} (*E. Merck*, Darmstadt). Solvent systems: TLC.-A: chloroform/5% methanol; TLC.-B: benzene/5% ethyl acetate; TLC.-C: benzene/15% ethyl acetate. Substances were eluted with ether or ether/10–20% methanol. For column chromatography, 200–325 mesh SilicAR CC-7 (*Mallinckrodt*, Saint Louis, Mo.) was used. Gas chromatograms were obtained on *Hewlett Packard* Models 5750 and 5710 A gas chromatographs (flame ionization detector). Columns: 180 × 0.6 cm glass; GLC.-A: 3% EGSS-X on Gas Chrom Q; GLC.-B: 3% XE-60 on Gas Chrom Q; GLC.-C: 3% OV-1 on Gas Chrom Q; GLC.-D: 15% Carbowax 20M on Chromosorb WAW, DMCS (all column packings from *Applied Science Laboratories*, State College, Pa.); carriergas: helium. Programmed runs were made with a heating rate of 4°/min after an isothermal postinjection period of 4 min.; peak areas were determined by planimetry. For high pressure liquid chromatography (HPLC), a Model ALC 202 Chromatograph (*Waters Associates*, Milford, Mass.) was used.

Radioassay. – Aliquots of all preparations were counted in 10 ml scintillation liquid (7 g PPO, 0.05 g POPOP and 100 g naphtalene in 1000 ml Dioxane, or 5 g PPO and 0.1 g POPOP in 1000 ml toluene) in a *Packard* Tricarb Model 3375 liquid scintillation spectrometer. The counting efficiencies were determined by the automatic external standardization method using butanone 2,4-dinitrophenylhydrazone quenched samples as standards. Occasionally the counting efficiency in a particular sample was double-checked by internal standardization (addition of [^{3}H]- or [^{14}C]-toluene). All samples were counted with a standard deviation of $\leq$ 2.0% [level of confidence: 95.5% (2σ)]. Estimated limits of error in the method: $\pm$ 5%.

Chemicals and performance of reactions. – The following solvents were used: ethyl acetate, methylene chloride, benzene, methanol (all nanograde, *Mallinckrodt*); chloroform (reagent grade, *Fisher*); ether (anhydrous, analytical reagent, *Mallinckrodt*); pyridine (distilled over barium oxide); dimethylformamide (fractionated after addition of 12% benzene and 5% water); tetrahydrofurane (freshly distilled from lithium aluminumhydride); water (distilled in a glass-apparatus). Solid chemicals and reagent solutions: inorganic chemicals were analytically pure; 3,5-dinitrobenzoyl chloride was crystallized from ether in an atmosphere of dry nitrogen, m.p.: 66–68°. Ruthenium dioxide (59.8% Ru) was purchased from *Engelhard Industries*, Newark, N.J. The phenylmagnesium bromide solution was obtained by filtration of the reaction mixture from phenyl bromide and magnesium in ether; its concentration was determined by titration. The reactions for the degradation of juvenile hormone were performed in KIMAX culture tubes with teflon coated screw caps. If not otherwise indicated, the reaction temperature was 25 ± 3°. 'Usual work up' means: extraction with 0.5–1 ml ether (3–6 times), washing the combined ether phases with water, saturated aqueous sodium chloride, or solutions indicated in the individual experiments, reextraction of the aq. phase with ether (twice), drying the combined ether phases on sodium sulfate and evaporation in a stream of dry nitrogen.

Incorporation of 1-[^{14}C]-propionate (cf. [11]). – 1 mCi 1-[^{14}C]-propionate (15 mCi/mmol, *New England Nuclear*, Boston, Mass.) was dissolved in 0.5 ml Insect *Ringer* solution and injected in the abdomens of ten 1–2 day old male *Hyalophora cecropia* moths. After an incubation time of 28 to 48 h, the insects were sacrificed. During the extraction about 100 μg *JH-I* was added to facilitate the separations; the hormones were isolated by a procedure similar to that described in [11]. Following chromatography of the crude extract on a 2.5 × 103 cm Sephadex LH-20 (*Pharmacia Fine Chemicals*, Uppsala) column, the JH.-fraction was purified by HPLC 0.8 × 60 cm EM-Gel Si-150 (*E. Merck*, Darmstadt) in methylene chloride/12.5% chloroform/0.05% water). *JH-I* and *JH-II* were separated by HPLC on 0.4 × 30 cm μ-Porasil (*Waters Associates*, Milford, Mass.) in hexane/2% ethyl acetate/0.03% 2-propanol.

Reference compounds. – *JH-I* (**1**): A mixture of **1** and its 2-(*Z*)-isomer was purchased from ECO-control, Inc. (*Cambridge*, Mass.) and resolved by HPLC in hexane/2.8% ethyl acetate/0.04% 2-propanol on a 0.23 × 300 cm column packed with porasil-T (*Waters Associates*, Milford, Mass.). **1** was 95 ± 5% pure as indicated by GLC.: GLC.-A: 170°; GLC.-B: 160°. ε_{220} = 13,800 (methanol) (*cf.* [8d]).

7-([^3H]-Ethyl)-*JH-I* (specific activity: 14.1 Ci/mmol) was purchased from *New England Nuclear* (Boston, Mass.). An aliquot containing 55,000 dpm was diluted with 130 μg unlabelled **1** and repurified by HPLC in the same system as described above for *JH-I*. Radiochemical purity: ≥ 95% (see[1])).

Butanone 2,4-dinitrophenylhydrazone (**14**) was recrystallized from ethyl acetate, m.p. 108–109°; ε_{360} = 21,900 (ethanol).

2-Phenyl-butan-2-ol (**15**) was synthesized from phenylmagnesium bromide and butanone in ether. It was purified by distillation (b.p. 98–100°, 12 Torr), column chromatography in chloroform/0.5% methanol, and TLC.-A (Rf ~ 0.3). Purity ≥ 98% as determined by GLC.-C, program 80–190°; GLC.-D, program 100–240°. – NMR. (CCl_4): 7.4 (*m*, 5 arom. H); 2.2 (br. *s*, OH); 1.9 (*q*, *J* = 7 Hz, H_2C(3)); 1.6 (*s*, H_3C(1)); 0.9 (*t*, *J* = 7 Hz, H_3C(4)). – MS.: 150 (2), M^+; 135 (5), $M^+ - CH_3$; 132 (15), $M^+ - H_2O$; 122 (9); 121 (100), $M^+ - C_2H_5$; 117 (23), $M^+ - H_2O—CH_3$; 115 (9); 105 (7); 103 (6); 91 (9), $C_7H_7^+$; 78 (8); 77 (14); 57 (8); 51 (8); 43 (6); 32 (14). – UV.: $\varepsilon_{257,5}$ = 215 (ether) (*cf.* [13]).

Methyl (2E,6E)-3,7-dimethyl-10-(3,5-dinitrobenzoxy)-2,6-decadienoate (7-nor-**17**) was prepared from *JH-III* by application of the reaction sequence described below for the degradation of *JH-I*. It was purified by TLC.-B (twice developed, Rf ~ 0.75), and by HPLC in methylene chloride/0.2% water on a 0.8 × 180 cm column packed with EM gel Si-150 (*E. Merck*, Darmstadt). Purity: ≥ 98% (the preparation contained 17% of the 2(*Z*)-isomer) as determined by GLC.-C, program 185–275°. – MS.: 420 (0.5), M^+; 402 (2), $M^+ - H_2O$; 389 (6), $M^+ - CH_3O$; 370 (5); 361 (7), $M^+ - COOCH_3$; 195 (13), $(O_2N)_2C_6H_3CO^+$; 173 (6); 149 (14); 147 (8); 145 (9); 139 (6); 115 (5); 114 (23), $C_6H_{10}O_2^+$; 107 (6); 96 (6); 95 (100), $C_6H_7O^+$; 93 (11); 85 (5); 83 (12); 82 (11); 81 (7); 79 (8); 75 (9); 71 (6); 69 (7); 67 (27); 57 (9); 55 (21); 53 (7); 43 (7); 41 (5); m*: 60.43 114 → 83); 47.25 (95 → 67). – UV.: ε_{228} = 28,100; ε_{300} = 396 (cyclohexane).

4-Oxo-hexanyl 3,5-dinitrobenzoate (**18**). The tetrahydropyranyl 3-bromopropyl ether (b.p.: 77–81°, 4 Torr) reacted with sodium cyanide in dimethyl sulfoxide (*cf.* [17]) to the tetrahydropyranyl 3-cyanopropyl ether (b.p.: 97–103°, 4 Torr), which was added to a solution of ethylmagnesium bromide in ether/tetrahydrofurane. The reaction mixture was decomposed with 4N hydrochloric acid in water/dioxane; the crude product was distilled. To the fraction with a boiling range of 80–95°/4 Torr, 3,5-dinitrobenzoyl chloride in pyridine was added. The mixture was stirred at 25° overnight. After addition of 2N sulfuric acid, the crude product was extracted with methylene chloride and purified by column chromatography in benzene/3% ethyl acetate. Some lower boiling impurities were distilled off in a *Kragenkolben* at 80°/0.5 Torr. The residue was resolved by column chromatography in benzene. The 4-oxo-hexanyl 3,5-dinitrobenzoate fraction was recrystallized from ether/hexane, m.p. 82–84°. Purity: ≥ 99% as indicated by GLC.-C, program 150–250°. – NMR. ($CDCl_3$): 9.1 (*m*, 3 arom. H); 4.49 (*t*, J = 6 Hz, $H_2C(1)$); 2.9–1.8 (*m*, $H_2C(2)$, $H_2C(3)$, $H_2C(5)$); 1.07 (*t*, J = 8 Hz; $H_3C(6)$). – MS.: 310 (0.2), M^+; 295 (0.4), $M^+ - CH_3$; 282 (12); 281 (5), $M^+ - C_2H_5$; 196 (10); 195 (100), $(O_2N)_2C_6H_3CO^+$; 165 (6); 149 (20), $O_2NC_6H_3CO^+$; 103 (6); 98 (17); 75 (19); 72 (37); 57 (82); 41 (5); *m** 113.85 (195 → 149). – UV.: ε_{222} = 24,200; ε_{300} = 415 (cyclohexane).

4-Phenyl-1,4-hexanediol (**19**) was prepared from 4-oxo-hexanyl 3,5-dinitrobenzoate and phenylmagnesium bromide in ether and purified by prep. TLC.-B (three times developed, Rf ~ 0.5), *Kugelrohr*-distillation at 110–120°/0.1 Torr, and a second TLC.-B. Purity: ≥ 98% as indicated by GLC.-C, program 120–220°. – MS.: 194 (nil), M^+; 176 (6), $M^+ - H_2O$; 165 (31), $M^+ - C_2H_5$; 148 (11); 147 (96), $M^+ - H_2O - C_2H_5$; 145 (18); 136 (7); 135 (60), $M^+ - C_3H_7O$; 132 (8); 117 (20), $M^+ - C_6H_5$; 115 (11); 106 (9); 105 (100), $C_7H_5O^+$; 91 (24), $C_7H_7^+$; 78 (9); 77 (41), $C_6H_5^+$; 65 (6); 57 (38); 51 (11); 41 (11). – UV.: $\varepsilon_{275.5}$ = 203 (methanol).

p-*Phenyl-phenacyl levulinate* (**20**) was prepared from levulinic acid and *p*-phenyl-phenacyl bromide in dimethylformamide. It was purified by column chromatography in benzene and recrystallized from methylene chloride/ether and methylene chloride/pentane, m.p. 93.5–95°. TLC.-C (Rf ~ 0.35). Purity: ≥ 98% as indicated by GLC.-C, program 220–270°. – MS.: 310 (8), M^+; 181 (100), $M^+ - C_6H_9O_3$; 153 (15), $C_{12}H_9^+$; 152 (20), $C_{12}H_8^+$; 151 (6); 99 (37), $C_5H_7O_2^+$; 43 (7). – UV.: ε_{285} = 24,600 (methanol).

Benzoic acid was sublimed at 70–80°/0.6 Torr. – UV.: ε_{272} = 854; ε_{279} = 691 (methanol) (*cf.* [14]).

Degradation of juvenile hormone (*cf.* [12])[2]. – *Methyl (2E,6E)-3,11-dimethyl-7-ethyl-10,11-dihydroxy-2,6-tridecadienoate* (**13**). 1.23 mg (4.19 μmol; 25,800 dpm ^{14}C) *JH-I* (**1**) in 0.5 ml tetrahydrofurane and 0.3 ml 0.014% perchloric acid was kept for 2 h in the dark. After the addition of 1 ml saturated aq. sodium chloride the mixture was worked up as usual. The combined ether extracts were washed with 1% aq. sodium hydrogencarbonate. The diol-ester **13** was purified by TLC.-A (Rf ~ 0.36). Yield: 1.08 mg (83%), 23,900 dpm (92%).

Butanone 2,4-dinitrophenylhydrazone (**14**) *from* **13**. To 2.12 mg **13** (6.79 μmol; 25,200 dpm ^3H; 16,400 dpm ^{14}C) in 1 ml methanol, 15 mg sodium metaperiodate in 1 ml water was added. After 2.5 h in the dark, 15 mg sodium thiosulfate was added and the mixture kept for another 15 min. Nitrogen was bubbled through the reaction mixture and through two traps in series. The traps were charged each with 1 ml 0.025M 2,4-dinitrophenylhydrazine in ethanol/95% phosphoric acid = 6/4. 10 ml KIMAX culture tubes served as reaction vessel and traps. They were sealed with silicone rubber septa and connected with stainless steel capillary tubing. After 3.5 h, the contents of the two traps were combined, diluted with 5 ml water and worked up as usual. The ether extracts were washed with saturated aq. sodium hydrogencarbonate. **14** was purified by TLC.-B on silicagel H (*E. Merck*, Darmstadt) (Rf ~ 0.55). The appropriate zone was eluted with ethanol[3]. Yield: 1.14 mg (67%); 0 dpm ^3H; 5786 dpm ^{14}C (35% ^{14}C).

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[2]) Selected representative experiments. All mass determinations by UV.-colorimetry.

[3]) Reagent blanks showed a background absorption at 360 nm, corresponding to 100 ± 50 μg (0.4 ± 0.2 μmol) butanone 2,4-dinitrophenylhydrazone which had to be subtracted for the determination of butanone. Since the yields of butanone 2,4-dinitrophenylhydrazone in the reactions were 2.5–3.7 μmol, the relative error in the determination of the specific activity of this derivative is in the range of ± 8%.

2-Phenyl-butan-2-ol (**15**) *and methyl* (*2E,6E*)*-3,7-dimethyl-10-*(*3,5-dinitrobenzoxy*)*-2,6-decadienoate* (**17**) *from* **13**. 1.03 mg **13** (3.29 μmol; 22,800 dpm ^{14}C) were treated with sodium metaperiodate as described above. Following addition of 8 mg (0.11 mmol) butanone, a stream of nitrogen was passed in sequence through the reaction mixture, a drying tube with powdered barium oxide[4]), and 2 ml of 0.5 M phenylmagnesium bromide in ether. The upper part of the trap containing the *Grignard*-reagent was chilled with dry ice. After 2.5 h the trap was sealed and kept in a water bath at 40–50° for 10 min. Decomposition of the reaction mixture with saturated aq. ammonium chloride at 0° and usual work up yielded crude 2-phenyl-butan-2-ol (**14**) which was obtained pure after TLC.-A, *Kugelrohr*-distillation at 75–80°/12 Torr, and a second TLC.-A. Yield: 4.22 mg (25% of added unlabelled butanone), 2750 dpm (12%).

The methanolic/aqueous solution remaining after transfer of the butanone was cooled to 0° and treated with 5–10 mg sodium borohydride. After 30 min. at 0° and 30 min. at 25° the hydroxy-ester **16** was extracted as usual and purified by TLC.-A (Rf ~ 0.44). GLC.-A: 170° and GLC.-B: 170° indicated a purity of ≥ 98%. 0.5 ml of 0.3 M 3,5-dinitrobenzoyl chloride in pyridine were added to the hydroxy-ester **16** in 0.1 ml pyridine. After 2 h the pyridine was evaporated in a stream of nitrogen, the residue worked up with 1 N sulfuric acid, methylene chloride, and 1% sodium hydrogencarbonate. After TLC.-B (Rf ~ 0.42), **17** was ≥ 99% pure as determined by GLC.-C. Yield: 1.04 mg (73%); 8450 dpm (37%).

Oxidative cleavage of **17** *with ruthenium dioxide/sodium periodate*. 1.00 mg (2.30 μmol; 8110 dpm ^{14}C) of the DNB-ester **17** in 0.1 ml t-butyl alcohol was combined with 0.5 mg ruthenium dioxide, 20 mg sodium metaperiodate and 0.7 mg periodic acid in 0.1 ml water. After 1 h 10 mg sodium metaperiodate in 0.3 ml water, after another h 12 mg sodium metaperiodate and 0.5 mg ruthenium dioxide in 0.1 ml water were added. After a total reaction time of 5 h, the reaction was terminated by addition of 0.1 ml 2-propanol. 1 ml water, 1 ml ether and 3 drops of saturated aq. sodium hydrogencarbonate were added and the mixture worked up as usual. TLC.-B of the ether extract gave *4-oxo-hexanyl 3,5-dinitrobenzoate* (**18**) (Rf ~ 0.29). Yield: 0.370 mg (52%); 4670 dpm (57.6%).

The aqueous phase was acidified with 2 N sulfuric acid and extracted continuously with ether for 8 h. The ether was evaporated and the pH of the residue (50–100 μl) adjusted to 8–9 (phenolphthaleine) with 1% aqueous sodium hydrogencarbonate. After addition of 0.5 ml dimethylformamide and 10–15 mg *p*-phenyl-phenacyl bromide the mixture was kept for 2 h at 20–25°. The solvent was evaporated in a stream of nitrogen at 50–60°; traces of dimethylformamide were removed by addition of methanol and repeated evaporation. The p-*phenyl-phenacyl levulinate* (**20**) was purified by TLC.-C (Rf ~ 0.35) and HPLC in methylene chloride/12.5% chloroform/0.05% water on 0.8 × 60 cm EM-Gel Si-150 (*E. Merck*, Darmstadt). Yield: 0.041 mg (6%), no detectable radioactivity (< 5 dpm in 0.021 mg).

4-Phenyl-1,4-hexanediol (**19**). 0.355 mg (1.15 μmol; 4480 dpm) 4-oxo-hexanyl 3,5-dinitrobenzoate (**18**) were diluted with 5.35 mg (17.25 μmol) unlabelled **18** and dissolved in 1 ml ether. After addition of 0.5 ml 1 M phenylmagnesium bromide in ether at 0°, the deeply brown colored mixture was heated for 15 min. to 100°, cooled to 0°, and decomposed with saturated aq. ammonium chloride. The reaction mixture was worked up as usual. **19** was purified by TLC.-A, distillation at 110–120°/0.1 Torr, and another TLC.-A. Yield: 3.07 mg (86%), 4050 dpm (90%).

Benzoic acid from 2-phenyl-butan-2-ol (**15**). 3.86 mg (25.7 μmol, 2520 dpm ^{14}C) 2-phenyl-butan-2-ol (**15**) in 1.5 ml *Kuhn-Roth*-acid [18] was heated for 15 min. to 100°. The solution was cooled to 0° and after terminating the reaction by addition of saturated aq. sodium hydrogensulfite worked up as usual. The combined ether phases were extracted with 2.5 ml 0.7 N sodium hydroxide in three portions, the alkaline aq. solution reextracted with ether, acidified with 2 N sulfuric acid and reextracted with ether. The benzoic acid was purified by sublimation at 70–80°/0.2 Torr. Yield: 1.80 mg (58%); 1390 dpm (55%).

Benzoic acid from 4-phenyl-1,4-hexanediol (**19**). The procedure for the oxidation of the 4-phenyl-1,4-hexanediol (**19**) was essentially the same as described in the foregoing experiment. From 2.76 mg (14.2 μmol; 3650 dpm) 4-phenyl-1,4-hexanediol (**19**) 0.96 mg (55%) benzoic acid containing 2010 dpm (55%) was obtained.

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[4]) No radioactive material was absorbed on the barium oxide (elution with ether).

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112. Photochemische Reaktionen

81. Mitteilung [1]

Notiz zur UV.-Bestrahlung von γ, δ-Epoxy-eucarvon und dessen Reaktivität gegenüber Botrifluorid-äthylätherat

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(10. III. 75)

Summary. UV.-irradiation ($\lambda \geqslant 327$ nm) of the α,β-unsaturated γ,δ-epoxy ketone **2** in pentane gives the isomers oxidoketone **3** and diketone **4**, in high yield. On treatment with $BF_3O(C_2H_5)_2$, **2** undergoes rearrangement to the diketone **4** and the isomeric lactone **8** and yields also the dimer **9**.

1. Einführung. – Der UV.-Bestrahlung von γ,δ-Epoxy-eucarvon (**2**), gewonnen durch Oxydation von Eucarvon (**1**) [2] mit 3-Chlorbenzopersäure[2]), gehen Versuche

[1]) Auszug aus der Diplomarbeit von *A. P. Alder,* ETH-Z 1974.

[2]) Während der Ausführung dieser Arbeit erhielten wir Kenntnis von einer Veröffentlichung von *Hart & Lavrik* [3], in der ebenfalls das Epoxid **2** beschrieben wird.