(ZK 68260). Binding was markedly reduced when the 15-hydroxyl group was omitted (ZK 64894). $PGF_{1\alpha}$ (without double bond in upper side chain) together with the phenoxysubstituents, also showed good binding, which was decreased when the double bond in the lower side chain was substituted with a methylene ring (ZK 65704). The binding affinity of the substances listed in tables 1 and 2 compared well with the abortifacient potency of these compounds.

Discussion. The development of prostaglandin analogues possessing a higher luteolytic activity is of considerable importance because of their potential for use in reproductive physiology [10,11]. Although the exact mechanism of action of the prostaglandins remain undefined, a first step may be binding to specific receptors. Receptors for $PGF_{2\alpha}$ have been identified in corpora lutea of various species including man [6]. Time-course studies on superovulated rat ovaries have shown that receptors for $PGF_{2\alpha}$ reached peak levels on days 7 and 9 of pseudopregnancy [12]. In this investigation, radioligand binding studies in rat corpora lutea were performed with $PGF_{2\alpha}$, on day 7 after HCG administration. Information about the structural requirements of $PGF_{2\alpha}$ for receptor binding was established by comparing displacement curves for a wide variety of $PGF_{2\alpha}$ derivatives. It was found that modifications in the carboxyl group at C-1 gave compounds with high affinity for the receptor in decreasing order of effectiveness as follows:- COOR > COOH > OH. Spacefilling substituents at C-15 reduced binding, but a lipophilic group at C-1 increased it, as in the case of the biphenyl ester, compound (ZK 56193), which was found to be about 10 times as potent as $PGF_{2\alpha}$ itself. The contribution of the various substituents to the intrinsic binding affintiy is additive. A number of compounds with additional substituents in the phenyl ring have found wide application in veterinary medicine [13,14].

The data obtained from the binding analysis generally compared well with the abortifacient potency in pregnant rats. $PGF_{2\alpha}$ induces abortion as a result of its luteolytic effect, together with a direct stimulation of the uterine musculature. $PGF_{2\alpha}$ derivatives that displayed high potency in receptor binding in vitro, were also potent in terminating luteal function and inducing abortion.

In conclusion, the binding characteristics of 16-phenoxy derivatives of $PGF_{2\alpha}$ to rat luteal membranes, as well as their abortifacient potency in pregnant rats have been demonstrated. Contribution of various functional groups to the binding activity was also elucidated. It is thus apparent that the knowledge of the structural requirements of each receptor would allow the development of new compounds with unique combinations of properties and possibilities for novel clinical applications.

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Forced synthesis of trace amounts of juvenile hormone II from propionate by corpora allata of a juvenile hormone III-producing insect

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Summary. Corpora allata of the cockroach *Diploptera punctata* normally synthesize only the isoprenoid juvenile hormone III (JH III). Only under extreme in vitro conditions (absence of carbon sources other than propionate) do they produce trace amounts of the homoisoprenoid JH II in addition to JH III. The specificity of the in vitro synthesis of JH III by *D. punctata* is thus consistent with the observed lack of homoisoprenoid JHs in this insect.
Key words. Juvenile hormone synthesis; propionate; homoisoprenoid; cockroach; corpora allata.

The synthesis of homoisoprenoid JHs by corpora allata from Lepidoptera is now well established [1]. All non-lepidopteran insect species studied so far produce exclusively JH III, as shown by analysis of the product of in vitro incubations of their corpora allata or by reliable assays of hemolymph and/or whole body titers of JH [1]. For instance, the corpora allata of the viviparous cockroach *Diploptera punctata* synthesize only the isoprenoid JH III in vitro [2] and only JH III can be detected in the hemolymph by GC-MS, the homoisoprenoid JH II, JH I or JH 0 being discriminated at least at the 5×10^4 level [3]. The specificity of JH homolog production is of considerable interest, because many examples of loose substrate specificity in enzymes of JH biosynthesis have been presented [1]. Propionate can serve as precursor of the higher

homologs of JH III [1], and propionate is incorporated with little dilution into JH II by *Manduca sexta* glands (molar incorporation ratio of 0.82)[4]. It was felt that propionate should be tested as potential JH precursor in a JH III-producing insect, because, as pointed out by Schooley and Baker [1], the failure to demonstrate homologous JH production in such an insect in past studies might have been caused by the experimental conditions favoring JH III synthesis. We report here that even under extreme in vitro conditions the corpora allata of *D. punctata* can produce only traces of homoisoprenoid JH (JH II) from added propionate.

Materials and methods. The viviparous cockroach *Diploptera punctata* was reared as described previously [5]. Day-5 mated females were used in our experiments because they provide high activity corpora allata. A minimal incubation medium [5] consisting of Hank's salts, phenol red, 25 mM HEPES at pH 7.2, MEM vitamin mixture and 2% Ficoll was supplemented with carbon sources (sodium acetate, sodium propionate, glucose, amino acids) and radiolabeled precursors as indicated. Amino acids are a mixture of 12 amino acids at concentrations recommended for Minimum Essential Medium (Gibco) [5]. [methyl-^{3}H]Methionine (200 mCi/mmol, New England Nuclear) at a final concentration of approx. 0.05 mM was used as mass marker for the JH produced [6]. [1-^{14}C]Propionate (58 mCi/mmol) was obtained from New England Nuclear. The JH produced was extracted with isooctane [7] and analyzed by HPLC using a Perkin-Elmer model 400 liquid chromatograph, with a Rheodyne 7125 injector and Perkin-Elmer LC 95 variable wavelength detector (4.5 µl flow cell). Reversed phase HPLC used a Supelcosil LC-18 column (250 × 4.6 mm, 5 µm particle size) and the solvent system acetonitrile/water (70 : 30) at 1.5 ml/min. Normal phase HPLC used a Supelcosil LC-Si column (150 × 4.6 mm, 5 µm particle size) with a solvent system hexane (propanediol saturated)/ether (96 : 4) at 1 ml/min. Reference JH I, II and III were obtained from Behring Diagnostics.

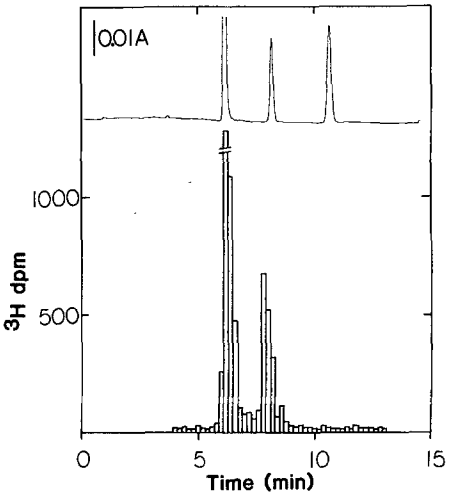
Results and discussion. We have recently shown that corpora allata incubated in the absence of carbon sources rapidly cease synthesizing JH III [5]. Table 1 shows that in the absence of other carbon sources, exogenous propionate is able to force the glands to produce JH II during the few hours of waning JH synthetic activity. Reversed-phase HPLC analysis of the secretion products of the corpora allata labeled from [methyl-^{3}H]methionine indeed revealed a radioactive peak less polar than JH III (fig.). This peak had a retention time which corresponded to that of authentic unlabeled JH II. The radioactivity associated with JH II on reversed phase HPLC had the same retention time as JH II on normal phase silica HPLC.

In order to demonstrate that propionate indeed served as precursor of JH II (or one of its isomers), we studied the incorporation of radioactivity from both [1-^{14}C]propionate and [methyl-^{3}H]methionine into JHs under conditions similar to those described above. [1-^{14}C]Propionate was chosen as label because the C2 and C3 carbons of propionate can be randomized in JH homologs [8], probably because of the metabolism of propionate to acetate with loss of the C1 carbon of propionate [9], whereas labeling from [1-^{14}C]propionate is specific into the homoisoprenoid portion of JH II and JH I [1]. Table 2 shows that only the JH II peak on reversed phase HPLC was labeled with both ^{3}H and

Table 1. Effect of propionate on JH synthesis. Corpora allata (8–10 pairs per experiment) from day-5 mated females were incubated for 5 h in a minimal medium containing only the carbon sources listed and [methyl-^{3}H]methionine at a concentration of 57–75 µM. The JH synthesized was extracted by isooctane partition and analyzed by reversed phase HPLC as described in 'Materials and methods'. The amount of radioactivity in the JH II peak is expressed as a percentage of the total radioactivity in the JH III and JH II peaks.

Sole carbon source of the medium	JH II (%)	Total JH produced (pmol/pair)
3 mM propionate	6.4 ± 2.6[a]	116
3 mM propionate + 5.5 µM glucose	3.4 ± 0.2	167
3 mM propionate + 5.5 mM glucose	1.6 ± 0.3	376
3 mM acetate	n.d.[b]	165
5.5 µM glucose	n.d.	199
Amino acid mixture[c]	n.d.	144
None	n.d.	104

[a] Mean ± S.E. of 3 separate experiments;
[b] n.d.: not detected (< 0.1%);
[c] including propiogenic amino acids, Val, Ile, Thr at 0.4 mM.



HPLC of the secretion products of corpora allata incubated in the presence of propionate as sole carbon source. Corpora allata from day-5 mated females were incubated for 5 h in a minimal medium containing 3 mM propionate and [methyl-^{3}H]methionine as only carbon sources. The JH synthesized was extracted by isooctane partition and analyzed by reversed phase HPLC as described in Materials and methods. Fractions were collected every 12 s between 4 and 13 min and assayed for radioactivity by liquid scintillation spectrometry. The JH III fraction out of scale was 34,828 dpm. In this experiment, the JH II peak was 4.2% of the total JH. The pattern of elution (UV absorbance at 225 nm) of reference JH III, JH II and JH I (in that order) is also shown at the top of the figure.

Table 2. Incorporation of radioactivity from [methyl-^{3}H]methionine and [1-^{14}C]propionate into JHs produced in the presence of 1.5 mM propionate as sole carbon source. Corpora allata (20 pairs) from day-5 mated females were incubated for 5 h and the products were extracted by isooctane partition and analyzed by reversed phase HPLC as described in 'Materials and methods'.

	JH III	JH II	% JH II	MIR[a] in JH II (^{14}C/^{3}H)
Experiment 1:				
^{3}H	128,211 dpm	6,468 dpm	4.8	
^{14}C	126 dpm	1,679 dpm		0.89
Experiment 2:				
^{3}H	63,883 dpm	3,526 dpm	5.2	
^{14}C	57 dpm	998 dpm		0.98

[a] MIR = molar incorporation ratio calculated using the specific activity of propionate (58 mCi/mmol) and of methionine (200 mCi/mmole). Recovery of injected ^{14}C and ^{3}H in the JHs was 78 and 89% respectively.

^{14}C, confirming the specific incorporation of propionate into JH II. Calculation of the molar incorporation ratio from [1-^{14}C]propionate using the ^{3}H label as mass marker for the JH produced [6] clearly showed that propionate was incorporated without significant dilution, thus showing that all the JH II produced was derived from the exogeneous propionate, and that the corpora allata did not produce significant amounts of propionate (propionyl-CoA) from endogenous sources.

These experiments show that the corpora allata of *D. punctata* could be forced to utilize both the exogenous propionate (as homomevalonate precursor) and the endogenous mevalonate precursors for the synthesis of JH II. However, even under these artificial conditions JH III remained the principal product of the corpora allata, and no JH II was produced in the absence of propionate. An amino acid mixture, including those amino acids that could theoretically be metabolized to propionyl-CoA, did not support the synthesis of JH II. The addition of increasing concentrations of glucose to the incubation medium drastically reduced the percentage of JH II detected, although the amounts of JH II synthesized remained relatively unaffected. The very low levels of JH II produced from propionate indicate a strong discrimination against JH III homolog precursors, possibly starting with propionyl-CoA synthesis from propionate (via acetyl-CoA synthetase).

The observation of trace amounts of JH II in incubations of corpora allata with 3 mM propionate and a glucose concentration (5.5 mM) which supports high levels of JH III synthesis (table 1) suggests that the absence of JH III homologs in *D. punctata* is due principally to the lack of an adequate supply of propionyl-CoA in the glands [5]. Propionyl-CoA could theoretically be produced from several amino acids (Met, Val, Ile, Thr) or from β-oxidation of uneven-length fatty acids. It can be hypothesized that Lepidoptera evolved a specialized mechanism for the generation of propionyl-CoA in the corpora allata.

In contrast to the higher molar incorporation ratio of propionate into JH II noted here and in *M. sexta* corpora allata [4], the entry of mevalonate and homomevalonate into the biosynthetic pathway is limited [1, 4, 5]. Nonetheless, the synthesis of JH II by isolated corpora allata from *M. sexta* and *Heliothis virescens* can be stimulated by the addition of homomevalonate to the incubation medium [1, 10]. Similarly, JH III synthesis can be stimulated under certain conditions by exogenous mevalonate in *D. punctata* [5, 11] and several other JH III producing insects [10]. It is perhaps not surprising that the corpora allata from *Blaberus discoidalis, Leptinotarsa decemlineata, Periplaneta americana, P. feluginosa,* and *Tenebrio molitor* were found to produce only JH III even when supplied with homomevalonate [10] and conversely, that the glands from male *Hyalophora cecropia* could not be forced to make JH III acid even when supplied with mevalonate [12]. This high level of discrimination against the precursor to the wrong JH homolog(s) may be caused principally by the poor penetration of the C_6 or C_7 precursors.

Results obtained with other intermediates in cell-free systems seem to indicate a lax specificity. Homogenates of corpora allata incubated with 0.4 mM of acetyl-CoA and propionyl-CoA produce hydroxymethylglutarate (HMG) and hydroxyethylglutarate (HEG) presumably as their CoA thioesters [1]. The ratio of HMG/HEG produced was lowest (1.1) in *Tenebrio molitor*, an insect that normally produces only JH III. Higher values were obtained in *Schistocerca nitens* (7.9) and *Manduca sexta* (4.9–8.4), whereas rat liver enzymes produced HMG and HEG at a ratio of 13.5–17.4 under these conditions [13]. The specificity of HMG-CoA production thus appears lower in insects than in vertebrates, and does not seem to be lowest in corpora allata that make homoisoprenoids as well as JH III. Surprisingly, corpora allata homogenates of *S. nitens* reduced HEG-CoA to homomevalonate 10 times better than the normal HMG-CoA to mevalonate conversion [13]. The synthesis of compounds with the JH 0, JH I or JH II skeletons by pig liver and silkworm farnesyl pyrophosphate synthetase supplied with the appropriate substrates has been reported [14, 15] although the specificity of the insect corpus allatum enzyme is unknown. The last enzymes of JH biosynthesis also appear to lack a high degree of substrate specificity. In *Locusta migratoria*, the O-methyl transferase accomodates many types of substrates other than farnesoic acid in vitro, including JH 0 and JH I acids [16], and the methyl farnesoate epoxidase can utilize precocene [17] as substrate, with well-documented cytotoxic consequences.

In conclusion, our results indicate that the complete absence of JH III homologs under physiological conditions is not the result of absolute specificity by any enzyme downstream from propionate; indeed, many of these enzymes show little or no discrimination against the higher homologs. Rather, our results focus attention on the failure to generate propionate or propionyl-CoA as the key determinant of the homolog specificity of corpora allata from lower orders.

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