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Isolation, Structure, and Absolute Configuration of a New Natural Insect Juvenile Hormone from *Manduca sexta*

(organ culture/corpora allata/high-resolution liquid chromatography/tobacco hornworm)

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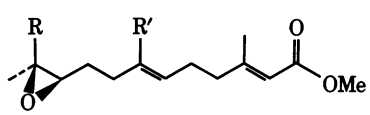
ABSTRACT **Two juvenile hormones are isolated from organ cultures of corpora allata of the tobacco hornworm moth, *Manduca sexta* Johannson, and are purified by high-resolution liquid chromatography. These are identified as methyl (2*E*, 6*E*)-(10*R*)-10,11-epoxy-3,7,11-trimethyl-2,6-dodecadienoate, a new natural hormone, and methyl (2*E*,6*E*) - (10*R*,11*S*) - 10,11 - epoxy - 3,7,11 - trimethyl-2,6-tridecadienoate. [^{14}C]Methionine is incorporated only into their methoxycarbonyl group. Details of the *in vitro* techniques and the chemical proof of structures are presented. The significance of the occurrence of a new juvenile hormone and the new techniques used for production, isolation, and identification are discussed.**

The chemical structures of two natural juvenile hormones 1 and 2 are known from studies of three species of giant silk moths, *Hyalophora cecropia* (1, 2), *Hyalophora gloveri* (3), and *Samia cynthia* (4). No information has been available on the question of diversification of juvenile hormone structures in other families of insects, since the extreme difficulties of obtaining sufficient quantities of purified hormone from animal homogenates for chemical analysis have discouraged extensive investigations.

Continued improvements in insect tissue culture techniques have led to their increased use in studies of endocrine physiology (5). Indirect indications have been obtained that corpora allata can survive and function *in vitro* (6), and recently cecropia juvenile hormone has been recovered and identified from cultures of brain-corpora cardiaca–corpora allata complexes of *H. cecropia* (7).

We report both an *in vitro* system that cleanly produces two hormones 2 and 3 from corpora allata of *Manduca sexta*, and the greatly simplified purification and chemical identification of the active hormones, one of them identified for the first time as a natural product of insects.



(1) R = R′ = Et
(2) R = Et, R′ = Me
(3) R = R′ = Me

MATERIALS AND METHODS

Animals. The *Manduca sexta* were laboratory reared in a continuous colony for many generations on an artificial diet (8). Under our conditions, 27°, 50% relative humidity, 16-hr photoperiod, *Manduca* completes a single generation in about 45 days.

Preparation of Cultures. Adult female moths, 0–48 hr after eclosion, were anesthetized with CO_2 and surface sterilized by immersion in 70% ethanol; the cuticular scales and hairs over the head and thorax were rubbed off with paper towel. The dorsal body wall over the head and cervical membrane was cut away, taking care not to bring the external surface into contact with the underlying tissues. The corpus cardiacum–corpus allatum complex was located attached to either side of the aorta posterior to the brain, and was removed by cutting the aorta, nerves, and tracheae in front of and behind the glands. Groups of excised glands were transferred through four successive baths of culture medium, the first two containing 1 mM recrystallized phenylthiourea, and then into a sterile Leighton culture tube containing 0.5 ml of fresh culture medium.

During incubation at 20–25°, the culture medium was replaced at 2- to 8-day intervals with a total of three changes over a 15-day period. The medium removed from the cultures was either extracted immediately or stored for up to 2 weeks at −20°.

Culture Medium. We used Grace's Insect T.C. Medium (GIBCO) without methionine, supplemented with 1% (w/v) bovine plasma albumin (fraction V, Metrix) and L-[*methyl*-^{14}C]methionine (ICN, 48.4 Ci/mol) to a final concentration of 0.04 mM, containing 2 μCi/ml.

Extraction Procedure. Culture media were extracted three times by vigorous shaking with equal volumes of highly purified ethyl acetate. The pooled organic phases were reduced to a small volume under N_2, applied to washed (hexane–ethyl acetate 1:1) thin-layer chromatography plates (silica gel GF-254, Brinkmann, 0.25 mm thickness), and developed in hexane–ethyl acetate 1:1 (v/v). Chromatoplates were radioscanned (Packard model 7201) and appropriate zones were eluted with pure ethyl acetate and stored in that solvent at −20°.

Chromatography and Spectroscopy. High-resolution liquid chromatography separations were performed on a homemade instrument incorporating a Haskel Engineering high-pressure pump, an LDC model 1205 UV-absorbance detector, and three 1 m × 2.4 mm inner diameter columns packed with Corasil II adsorbent (Waters Associates) connected in series.

Gas-liquid chromatographic analyses were performed on a Hewlett-Packard model 402 chromatograph with 2 m × 3

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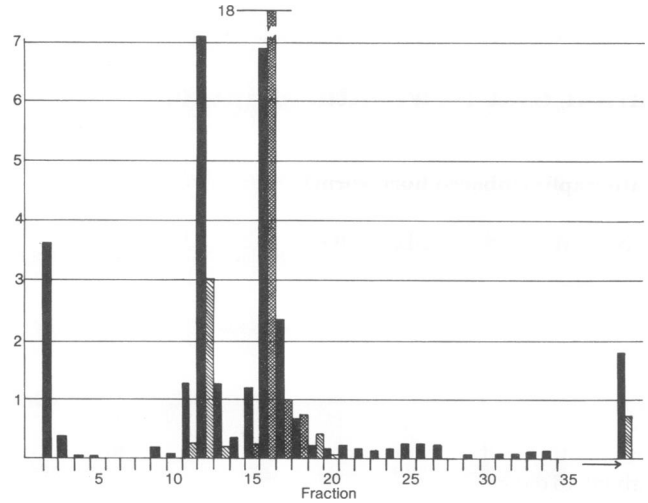

FIG. 1. Micropreparative gas-chromatographic fractionation of a tissue culture medium extract eluted from the "hormone-zone" after thin-layer chromatography. Two distinct regions are noted in which the radioactivity and biological activity are coincident. Although the amounts of radioactivity are nearly equal in these two regions, the later-eluting region (fractions *15–17*) contains the greater amount of biological activity. *Solid bar*, cpm $\times 10^{-4}$; *hatched bar*, units $\times 10^{-3}$; *double-hatched bar*, units $\times 10^{-4}$.

mm inner diameter glass columns packed with 3% phenyldiethanolamine succinate on silanized, acid-washed Chromosorb. For coupled gas chromatographic/mass spectral studies, an identical chromatograph was interfaced to a Varian MAT CH-7 mass spectrometer with a novel single-stage silicone membrane separator mounted directly in the column oven.

Ultraviolet spectra were measured on a Hitachi Perkin–Elmer model 124 spectrophotometer with a 1-mm path length cell, using Mallinckrodt spectrograde methanol.

Radioactivity was measured on a Packard Instruments model 3380 scintillation counter using a solution of toluene–2-methoxyethanol 2:1 containing 0.55% PPO.

Solvents. Ethyl acetate and pentane (Mallinckrodt) were rigorously purified by standard methods (9); this purification was mandatory. As a precaution, ether and *n*-hexane (Mallinckrodt nanograde) were redistilled before use.

Bioassay. Juvenile hormone activity was determined by

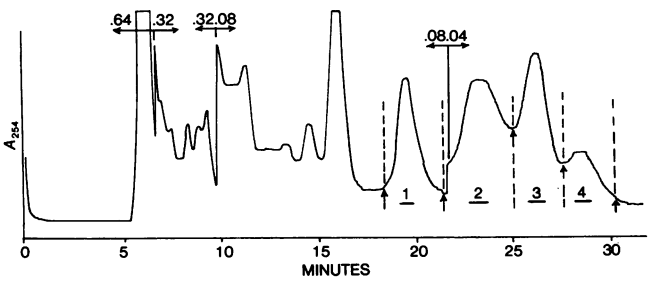

FIG. 2. Liquid chromatographic separation of pooled culture medium extracts (purified by thin-layer chromatography). Changes in the attenuation of the UV detector are noted by double-headed *arrows*. Four peaks (*1–4*) were collected from the region having the approximate retention volume of known juvenile hormones.

means of the *Galleria* wax test (10). Under our conditions, both cecropia hormones give a 50% response in treated *Galleria* at a concentration of about 5 pg per pupa. A similar test has been conducted in a few cases with 0–48 hr old *Manduca* pupae.

RESULTS

Initial cultures of adult female cardiacum–allatum complexes yielded an ethyl acetate-extractable, labeled material that migrated on thin layer in the same region as cecropia hormone. Elution and bioassay of separate zones indicated the presence of biologically active material only in this zone. Control cultures of medium alone, muscle, fat body, brain, or ovarioles from adult female moths produced no such radiolabeled material and were biologically inactive. In a few cases, cultures prepared in a *defined* medium (Grace's without supplement) also incorporated label, which migrated with juvenile hormone on thin-layer chromatograms.

Although up to 10-fold differences existed between comparable cultures, the glands during the first incubation typically produced 3000–3500 dpm of purified labeled material per gland pair per day. During the second incubation about 2000–2500 dpm—and during the third about 1000–1500 dpm—per gland pair per day was produced.

This general rate of production was unchanged by addition of fat body to the cultures, culture of intact brain-cardiaca–allata complexes, culture of the corpora allata alone (without cardiaca or aorta wall), or by cutting the allata to expose the internal cells directly to the medium. However, homogenization of allata in a glass tissue grinder destroyed their ability to incorporate the label.

After incubation in labeled medium, more than half the glands maintained for up to 100 days in unlabeled Grace's medium retained the ability to elicit a positive hormonal response when implanted into *Galleria* pupae.

Surprisingly, corpora allata from young adult male *Manduca* and *H. cecropia* moths failed to produce any biologically active or labeled product under these conditions. Cultures of brain-cardiaca–allata complexes from early fourth-instar *Manduca* larvae were generally inactive, but a single culture of corpora allata alone from animals at this stage produced a relatively small amount of labeled, biologically active ma-

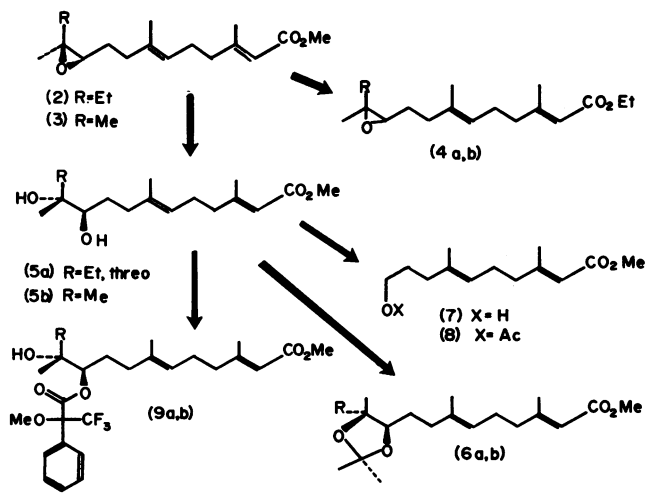

FIG. 3. Flow diagram of the microdegradative reactions used to identify the two *Manduca* hormones. *Manduca* hormone I is compound **3** and *Manduca* hormone II is compound **2**.

terial that migrated on thin layer in the same region as the product of the adult glands.

To demonstrate the presence of ^{14}C within the biologically active component of the extract, a sample was preparatively fractionated by gas–liquid chromatography. Aliquots of the 35 collected fractions were assayed for radioactivity and biological activity. Two distinct regions were resolved, in which most of the label and most of the biological activity were coincident (see Fig. 1). One of the regions coincided in retention time with the $C_{17}H_{28}O_3$ cecropia hormone (2). The other region was clearly *not coincident* with the cecropia hormones and was subsequently shown to have the same retention time on two different gas chromatographic columns as synthetic **3** ($C_{16}H_{26}O_3$) (11, 12).

In order to obtain a sufficient quantity of the unknown compounds for detailed chemical analysis, a series of 179 cultures containing 738 gland pairs was prepared over 3 months. A total of 209 ml of culture medium was collected in small batches, extracted, thin-layer chromatographed, and stored in ethyl acetate at −20°. The final pool contained 16.5×10^6 dpm of radioactivity and 2.8×10^6 *Galleria* units ("units") of biological activity.

Chemical Analysis. The pooled extract (16.5×10^6 dpm) was purified by liquid chromatography with 6% ether in pentane. This technique afforded nearly quantitative recovery and provided resolution about five times better than conventional thin-layer chromatography.

The chromatogram shown in Fig. 2 shows many fast-eluting (less polar) impurities and four UV-absorbing peaks eluting in the "hormone zone." The latter peaks comprised 9.58×10^6 dpm, or 58% of the injected radioactivity. The balance of the injected radioactivity was recovered in the forerun (1.07×10^6 dpm) (compounds less polar than the hormones) and in the afterrun and ether washes of the column (4.78×10^6 dpm) (compounds considerably more polar than the hormones). A total of 93% of the injected radioactivity was recovered.

Subsequent thin-layer separation of the polar material showed at least four labeled substances, whose R_F values were such that they should have been eliminated by the initial thin-layer purification. This pattern is typical of storage decomposition observed with synthetic juvenile hormone. Since this polar material possessed negligible biological activity (6.7×10^3 units), it was not further investigated.

The forerun, containing many impurities, possessed appreciable biological activity (625×10^3 units) and was therefore subjected to further liquid chromatography with a weaker solvent (4% ether in pentane). The radioactivity was clearly not coincident with the biological activity, so neither material was further investigated.

The remaining material of interest from the "hormone zone" comprised fractions *1–4* of Fig. 2. Each peak was separately rechromatographed (liquid chromatography), and aliquots were counted and bioassayed (see Table 1).

Peaks *2* and *4* had identical retention volumes to synthetic standards of compounds **2** and **3**, respectively. Because of the *combination* of low radio- and biological activity, peaks *1* and *3* were not further examined.

Analysis of 0.5 or 1% aliquots of each sample by gas chromatography revealed peak *2* to contain 85% of a compound (*Manduca* hormone II) having identical retention time to synthetic **2**, and 15% of a single impurity; peak *4* consisted of 93% of a material (*Manduca* hormone I) having identical retention time to synthetic **3**, and 7% of a single impurity.

TABLE 1. *Biological and radioassay of juvenile-hormone zone peaks after second liquid chromatography step*

Peak No:	1	2*	3	4*
dpm ($\times 10^6$):	0.12	4.10	0.24	3.19
Biological Activity (units):	$<1 \times 10^3$	2.3×10^6	5.3×10^3	2.3×10^4

* Careful rechromatography of each of these yielded 3.05×10^6 dpm of peak-*2* material and 1.98×10^6 dpm from peak *4*.

UV spectra were recorded with the entire sample of each substance in about 100 μl of methanol. Each hormone showed a UV maximum at 219 nm, with an extinction coefficient of 1.80×10^4 for *Manduca* hormone I and 1.72×10^4 for *Manduca* hormone II. Mass estimates used in calculating extinction coefficients were based on the mass spectrometrically determined isotopic purity of the two hormones (see below) and the observed dpm for the entire sample just before measurement of the UV spectrum, together with the known constant for the specific activity of isotopically pure ^{14}C. The values so obtained were 5.3 μg of *Manduca* hormone I and 8.7 μg of *Manduca* hormone II.

The solvent was removed and the residue was taken up in a few μl of redistilled nanograde hexane; about half of each sample was analyzed by gas chromatography–mass spectrometry. The mass spectra of *Manduca* hormones I and II were identical to reference spectra of unlabeled authentic samples (13) of **2** and **3**, respectively, except for those peaks retaining the *methyl* carbon of the *ester* moiety. The complete absence of m/e 116 peaks in our mass spectra of standards of **2** and **3** allowed good analysis of the ^{14}C content in the labeled hormones, which show both m/e 114 ($C_6H_{10}O_2^+$) and m/e 116 (14). *Manduca* hormone I showed a m/e 116/114 ratio of 44:117, or 73 atom % ^{14}C, while *Manduca* hormone II showed a m/e 116/114 ratio of 56:136, or 71 atom % ^{14}C.

Microchemical Analysis. In order to confirm the structure and to derive stereochemical information not obtainable from the spectral data in hand, several microchemical derivatization procedures were applied to tracer quantities of the labeled hormones diluted with appropriate unlabeled synthetic carriers. [The carrier for *Manduca* hormone II was a mixture (13) of epoxide isomers, about 80% *cis* and 20% *trans*.]

Reactions were usually conducted with 5,000–200,000 dpm (12–500 ng) of *Manduca* hormone and 50–100 μg of "cold" carrier. After solvent removal under reduced pressure at 25°, the reaction products were chromatographed on 5 × 20 cm silica GF plates washed with MeOH, then scanned. Each labeled zone was eluted with redistilled tetrahydrofuran; after solvent removal, the product was dissolved in appropriate solvents, injected onto the liquid chromatography column, and collected in scintillation vials. Coincidence of radioactivity with the UV-absorbance of carrier can be demonstrated with about 10 times the resolution of thin-layer chromatography with radioscanning.

All six of the following derivatives and degradation products (see Fig. 3) of each hormone showed unequivocal coincidence

on both thin-layer and liquid chromatography with their respective carriers by this method.

Transesterification to Ethyl Esters. Each hormone was stirred with ethanolic 0.1 M sodium ethoxide for 18 hr at 25° under argon. NMR and liquid chromatographic analysis of the product showed essentially quantitative conversion to the unlabeled ethyl ester with an 82/18 Δ^2 *trans* to *cis* ratio.

Epoxide Hydration. A clean and quantitative conversion to diols **5a** and **5b** was achieved by treatment of the stated quantities of diluted hormone with 0.02 M $HClO_4$ in tetrahydrofuran–H_2O 5:3 for 3 hr at 25°. In the case of *Manduca* hormone II, all label elutes only with *threo* diol **5a** derived from the racemic *Z*(*cis*) epoxide carrier, this diol being cleanly separated from the *erythro* isomer on liquid chromatography.

Optical Resolution and Absolute Configuration. Treatment of either diol **5a** (or **5b**) with the acid chloride of (+) α-methoxy-α-trifluoromethylphenylacetic acid (15) (0.1 M in pyridine) for 2 hr at 25° gave two diastereomeric esters **9a** (or **9b**) derived from cold carrier that could be completely separated by liquid chromatography. For each diastereomeric mixture, all radioactivity cochromatographed with the faster-eluting diastereomer, demonstrating the *optical activity* and 95–100% *optical purity* of each hormone.

In a separate experiment, the absolute configuration of the new hormone **3** was determined by correlation with an authentic sample (16) of (−) methyl (2*E*, 6*E*)−(10*S*)−10,11-dihydroxy-3,7,11-trimethyl-2,6-dodecadienoate (antipode of **5b**) of known absolute stereochemistry (17). The same batch of (+) α-methoxy-α-trifluoromethylphenylacetic chloride was used to convert this diol to its mono-ester. Liquid chromatographic examination of this derivative showed the predominant (75%) diastereomer to be the slower-eluting (unnatural) one, establishing the absolute configuration of natural **5b** as 10*R*.

Cleavage and Reduction of Diols **5**. Each diol was treated with 350 μl of 0.03 M $NaIO_4$ in 85% aqueous methanol for 0.5 hr, followed by reduction with 0.3–0.4 M $NaBH_4$ in ethanol for 0.5 hr. Each hormone gave, in about 80% yield, the same hydroxy ester **7**.

Acetylation of Alcohol Derivative **5**. Treatment of the previous derivatives **7** with pyridine–acetic anhydride 2:1 for 1.8 hr at 25° gave identical acetates **8**.

Acetonide Formation. Each diol **5** was stirred in a 0.02–0.04 M solution of *p*-toluenesulphonic acid in acetone for 0.5 hr at 25°. More than 90% formation of acetonide **6** was noted, and for *Manduca* hormone II separation of *erythro* and *threo* diol acetonides was observed, with radioactivity again coinciding with the *threo* isomer **6a**.

Hemolymph Analysis. In order to establish the presence of *Manduca* hormone I in blood of that species, 17.2 ml of hemolymph was collected from 144 early fourth-instar larvae and extracted with ether–ethanol 6:1. The extract was hydrolyzed in aqueous $HClO_4$ to convert epoxides to their corresponding 10,11-diols and thin-layer chromatographed against synthetic **5b**; the appropriate zone was eluted. The bis(trifluoro-acetyl)- derivatives were prepared by reaction of the diol with trifluoroacetic anhydride, the products were separated on thin layers and the region corresponding to identically derivatized synthetic **5b** was collected. The natural derivative was analyzed by gas chromatography with an electron-capture detector; it cochromatographed with the derivative of synthetic **5b** on two liquid phases of differing polarity. Attempts to verify the presence of *Manduca* hormone II ($C_{17}H_{28}O_3$) or $C_{18}H_{30}O_3$ in blood gave inconclusive results.

DISCUSSION

Through the use of novel organ culture, purification, and analytical techniques, we are able to report the identification of two juvenile hormones from the Sphingid moth, *Manduca sexta*. The first of these, methyl (2*E*,6*E*)-(10*R*)-10,11-epoxy-3,7,11-trimethyldodeca-2,6-dienoate ($C_{16}H_{26}O_3$), is a new natural hormone whose racemic form was described in 1965 as a synthetic mimic of the natural hormone (12). The second *Manduca* hormone is methyl (2*E*,6*E*)-(10*R*,11*S*)-10,11-epoxy-3,7,11-trimethyltrideca-2,6-dienoate ($C_{17}H_{28}O_3$), previously reported as one of two juvenile hormones found in cecropia and other Saturniid moths (2–4). We found no indication of the presence of the predominant cecropia juvenile hormone ($C_{18}H_{30}O_3$) in *Manduca sexta*.

Structure elucidation is based exclusively on hormones produced *in vitro* by corpora allata cultures, but the presence of $C_{16}H_{26}O_3$ *in vivo* has been confirmed in extracts of larval hemolymph. The biological activity of both of the *Manduca* hormones has been demonstrated by the classical *Galleria* wax test bioassay, as well as by tests on *Manduca* pupae.

Isolation of the hormones in a substantially pure state from culture media required only two proceduces after extraction, thin-layer followed by micropreparative liquid chromatography gave the two hormones in 85 and 93% purity. This extremely simplified purification scheme is attributable to the novel combination of tissue culture, liquid chromatography, and radiolabeling.

Ultraviolet spectra of the two isolated hormones showed maximum absorption at an identical wave length to standard samples of the Δ^2-*trans* hormone, but with a somewhat higher extinction, probably attributable to the single impurity contained in each hormone.

Microchemical derivatizations of the radiolabeled hormone allowed assignment of the label location and definition of olefin geometry and epoxide stereochemistry (not derivable from spectral data) with consumption of less than microgram amounts of the precious natural hormones. Thus, transesterification of both hormones with ethoxide ion showed complete loss of label, defining the sole location of methionine-derived ^{14}C to be the methyl in the ester moiety, in agreement with the findings of Metzler *et al.* (18). Acid hydrolysis of each hormone again supported the identity of the natural radioactive hormones with the respective synthetic carriers and, in the case of the presumed $C_{17}H_{28}O_3$ hormone, this experiment allowed assignment of the stereochemistry of the epoxide to be *Z*(*cis*), since all radioactivity cochromatographed only with the *threo* glycol formed from the synthetic *Z* epoxide. This observation was further supported by the behavior during liquid chromatography of the acetonides of the glycols derived from each hormone.

Cleavage of the epoxide followed by reduction of the resultant aldehydes from both hormones gave a common labeled fragment, with the same observation after acetylation of the reduction products. Thus, the only structural difference between the two hormones could be in the few carbon atoms attached to one side of the epoxide moiety.

The optical activity of the two hormones was demonstrated and the absolute configuration was determined by a

novel chromatographic resolution of diastereomeric esters formed from the spiked racemic diol derivatives of each hormone and the acid chloride of (+) α-methoxy-α-trifluoromethylphenyl-acetic acid. Thus, liquid chromatograms of these *mono*-esters showed equal amounts of the two carrier diastereomers, but the *radioactivity* coincided only with the faster-eluting diastereomer for both hormones. This demonstration of the optical activity and complete optical purity (within a 95–98% detection limit) of each hormone consumed about 50 ng of labeled material, a sensitivity, in this case, far greater than optical methods. Most important, the demonstration of optical activity points unequivocally to the biosynthetic origins of these *in vitro* hormones. An authentic sample (17) of the 10*S* antipode of **5b** gave an ester **9a** consisting predominantly of the slower-eluting diastereomer, found to be the *unnatural* diastereomer in the previous experiment. Since diols form from juvenile hormone epoxides with *retention* of configuration at C-10 (19), hormone **3** has absolute configuration 10*R*. Also, the same relative order of elution of the "natural" ester diastereomers from the two *Manduca* hormones points to identical 10*R* absolute configurations for each. Since *Manduca* hormone II has a *Z*(*cis*) epoxide stereochemistry, it follows that the configuration at its second center of asymmetry is 11*S*. These absolute configurations are the same as noted for cecropia hormone I (19, 20).

As a last step in the structure determination, about 30–50% of each sample was consumed for gas chromatography–mass spectral studies. Excellent mass spectra were obtained, identical to those of synthetic standards save for those peaks attributable to fragmentations in which the ester methoxyl was retained. The very high ^{14}C content of the hormones was determined from the m/e 116/114 ratio, since molecular ions from these hormones were visible, but weak. The values so obtained were 73 and 71 atom % ^{14}C for the methoxyl carbon in the isolated *Manduca* hormones I and II, respectively; values notably close to the 78 atom % isotopic purity of the highest specific activity batch of methionine added to culture medium. This incorporation rate of 90–100% implies a negligible endogenous methionine pool in the cultured tissues, and presumably a direct biosynthetic pathway from methionine to juvenile hormone probably involving *S*-adenosylmethionine, by analogy with bacterial methyl ester formation (21). Since no methionine is incorporated in the "isoprenoid" chain of the $C_{17}H_{28}O_3$ hormone (18), its biosynthetic origin remains obscure.

The results presented here provide the most direct evidence to date that corpora allata produce juvenile hormones. Röller and Dahm (7) showed that adult male cecropia brain–corpora cardiaca–corpora allata complexes cultured in a medium containing "inactivated" hemolymph produced $C_{17}H_{28}O_3$ and $C_{18}H_{30}O_3$ in about the same 1:5 ratio as found *in vivo*. In contrast, we have shown that *Manduca* $C_{16}H_{26}O_3$ and $C_{17}H_{28}O_3$ are produced in roughly equal quantities, and in cultures containing only corpora allata. Furthermore, we have observed hormone production in medium devoid of insect serum and in a few cases, a labeled material identical to juvenile hormone on thin-layer chromatograms has been produced by glands cultured in a defined medium lacking macromolecules. We are unable to explain the fact that under our conditions, adult male cecropia corpora allata produce no detectable hormone.

The technique of *in vitro* gland culture for studies of juvenile hormone structure and biosynthesis offers three significant advantages over the alternative technique of whole-animal extraction. First, the amount of hormone obtainable is up to 100 times greater per animal; second, the isolation from culture medium is many times more efficient than from whole-animal homogenates; and, finally, a radiolabel may be more readily introduced into hormone produced *in vitro*. Additionally, the hormone produced *in vitro* appears to be in solution, possibly bound to protein, making the authentic, biosynthesized hormone available for *in vitro* studies on target organs.

Finally, the actual physiological role played by the two *Manduca* hormones is not known. *Galleria* wax test bioassay indicates that *Manduca* hormone II is about 100 times more active than *Manduca* hormone I, although preliminary wax test assays on *Manduca* indicate that the difference between the two may be less in the homologous test. It is possible that the two molecular species stimulate different responses, although there is no direct evidence for such diversification. It is of greater importance that a single organ in culture produces both a true terpene hormone **3** and an homologous terpenoid **2**.

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