lowing them to ingest CE virus from gauze pledgets. When these mosquitoes were tested for CO_2 sensitivity we found that all the CO_2-sensitive mosquitoes were infected with CE virus; however, both +/− and misclassified mosquitoes were more common than they had been in mosquitoes infected by intrathoracic inoculation (Table 1). One possible explanation for the variation was that the mosquitoes received a relatively low dose of virus from the gauze pledgets. Kramer *et al.* (*9*) have shown that infection with western equine encephalomyelitis virus may be restricted to the midgut in mosquitoes that feed on low infectious doses. Thus, mosquitoes that became infected after ingesting a low viral dose would be nonsensitive to CO_2 if the virus had failed to escape the midgut and infect the organ system responsible for CO_2 sensitivity. Studies on sigma virus have shown that infection of the thoracic ganglion is necessary before CO_2 sensitivity will develop (*10*). A second possible explanation is that CO_2 sensitivity may vary over a range of incubation periods. In the present study incubation periods ranged from 9 to 28 days. Nearly all the mosquitoes inoculated intrathoracically were CO_2-sensitive 4 to 10 days after inoculation, but 9 out of 44 tested at 15 or more days were +/−, and 8 out of 42 tested at 17 or more days were nonsensitive. Thus, the +/− and nonsensitive mosquitoes might have been sensitive if they had been tested earlier.

Bussereau (*4*) found that rhabdovirus infections in *D. melanogaster* fell into two groups with respect to their effect on CO_2 sensitivity. The first group included sigma, SVC, PFR, and Piry virus infections that produced lifelong sensitivity to CO_2; the second group included infections caused by the VS serotype (Argentina, Brazil, Cocal, Indiana, and New Jersey) and Chandipura viruses that produced sensitivity after an appropriate incubation period. However, if the flies infected with VS or Chandipura viruses were held sufficiently long before they were exposed to CO_2, they reverted back to a nonsensitive state. This situation was observed with CE virus.

Sensitivity to CO_2 was not observed in transovarially infected progeny of female *A. dorsalis* or *A. melanimon* that had been infected with CE-WT virus by intrathoracic inoculation. No explanation can be offered for their lack of sensitivity.

It was important to determine if the CO_2 sensitivity was due to infection with CE virus or to the presence of a sigma-like agent in the CE-WT virus suspension. Since it was unlikely that field-collected mosquitoes would all contain the same contaminant, we tested individually the isolates of CE virus from three separate pools of collected *A. melanimon* (each isolate was passed one time in *A. dorsalis*). All of these isolates produced CO_2 sensitivity. As a further test, mouse antiserum to a plaque-purified clone of the BFS 283 strain of CE virus was used to neutralize CE-WT virus. Mosquitoes inoculated with CE-WT virus alone became infected and developed CO_2 sensitivity, while those inoculated with neutralized CE virus or with antiserum alone were neither infected nor CO_2-sensitive (Table 2). Thus, CO_2 sensitivity appears to be related specifically to infection with CE virus.

Table 2. Carbon dioxide sensitivity in *Aedes dorsalis* 7 days after inoculation with CE virus or CE virus plus CE antiserum. Infection status was determined by plaque assay in Vero cells.

Inoculum	Infection status	CO_2-sensitive	
		Yes	No
CE virus	+	10	0
CE virus plus CE antiserum	0	0	15
CE antiserum	0	0	8

When groups of infected and uninfected *A. dorsalis* and *C. tarsalis* were anesthetized with chloroform all the mosquitoes recovered, indicating that the sensitivity produced by CE infection is related to the CO_2.

The following questions remain to be answered. (i) Do other diptera infected with CE virus become CO_2-sensitive? (ii) Does CO_2 sensitivity extend to other members of the CE serogroup and to other bunyaviruses? (iii) Why were transovarially infected mosquitoes not CO_2-sensitive? (iv) What is the mechanism of CO_2 sensitivity in mosquitoes?

While this manuscript was in preparation, Rosen (*11*) reported results which extended the range of arthropods which become CO_2-sensitive following infection with a rhabdovirus from *Drosophila* to mosquitoes. The current study further extends our knowledge of virus-induced CO_2 sensitivity in arthropods by demonstrating that such sensitivity can be produced by a virus of the family Bunyaviridae and can occur in the natural mosquito vectors of CE virus, *A. melanimon* and *A. dorsalis* (*12*).

MICHAEL J. TURELL
JAMES L. HARDY
Naval Biosciences Laboratory and
Department of Biomedical and Environmental Health Sciences, School of Public Health, University of California, Berkeley 94720

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Juvocimenes: Potent Juvenile Hormone Mimics from Sweet Basil

Abstract. *Two compounds with highly potent juvenile hormone activity were isolated and identified from the oil of sweet basil,* Ocimum basilicum *L., Labiatae.*

During investigations of plants as sources of molecular models to implement the development of biorational chemicals for insect control we found that a commercial distillate of the herb sweet basil (*1*) possessed juvenile hormone activity. From 150 g of distillate we isolated by high vacuum distillation and subsequent open column and high performance liquid chromatography about 0.75 mg of each of two substances which were active in the milkweed bug juvenile hormone test (*2*) in the picogram range. The mass spectra of the unknown compounds designated **1** and **2** gave molecular ions at mass to charge (*m/e*) 282 and 298, respectively (*3*).

The nuclear magnetic resonance (NMR) spectra of compound **1** exhibited 26 protons and indicated the presence of *p*-methoxycinnamyl, isobutenyl, and methylbutadienyl moieties (*4*). These three visualized fragments appeared to be attached to a single methine carbon atom suggesting the provisional structure **1** shown in Fig. 1 (*5*). The NMR

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spectrum of compound **2** was very similar to that of compound **1** except for the presence of a 1,2-epoxy-2-methylpropyl moiety instead of an isobutenyl group (*6*) giving the structure **2** in Fig. 1. In further support of these structural assignments, the skeletal compounds (in Fig. 1, **3**, M^+, *m/e* 290, and **4**, M^+, *m/e* 304) were prepared from compounds **1** and **2** by catalytic hydrogenation (*7*). The physical constants (gas chromatography–mass spectrometry and NMR) of each compound were identical with those of compounds **2** and **4** synthesized by the procedure given in Fig. 1 (*8*).

From these data the structures of the hormonally active compounds from the sweet basil distillate are **1** and **2**. Since these compounds possess exceedingly high juvenile hormone activity, are derived from the plant *Ocimum basilicum*, and contain ocimene as a portion of their chemical structure, we have named them juvocimene **1** and **2** to correspond to the compounds given in Fig. 1 as **1** and **2** (*9*).

The juvocimenes appear to have resulted from the condensation of a monoterpene and a cinnamyl moiety. This speculation is supported by the presence of β-ocimene, methylchavicol, and methyl cinnamate as important constituents of the sweet basil oil distillate.

Throughout, purification activity was followed by applying the material topically in 1 μl of acetone to newly molted last instar nymphs of the milkweed bug *Oncopeltus fasciatus*. Supernumerary molting to giant nymphs gave evidence of high activity whereas nymphal-adult intermediates signaled less active fractions (Fig. 2). The dose response of the juvocimenes is compared with juvenile hormone **1** in Fig. 3. Juvocimene **2** induces the formation of nymphal-adult intermediates at treatment levels as low as 10 pg. Juvocimene **1** is approximately ten times less active than juvocimene **2**, although both plant-derived hormones are several orders of magnitude more active than the natural juvenile hormone **1** (*10*).

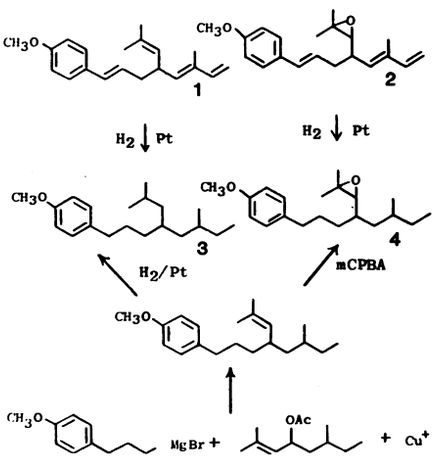

Fig. 1. Juvenile hormone mimics isolated from sweet basil distillate. Structural assignments are supported by the preparation of the skeletal compounds **3** and **4** from **1** and **2** by catalytic hydrogenation or synthesis (or both) as shown (*8*).

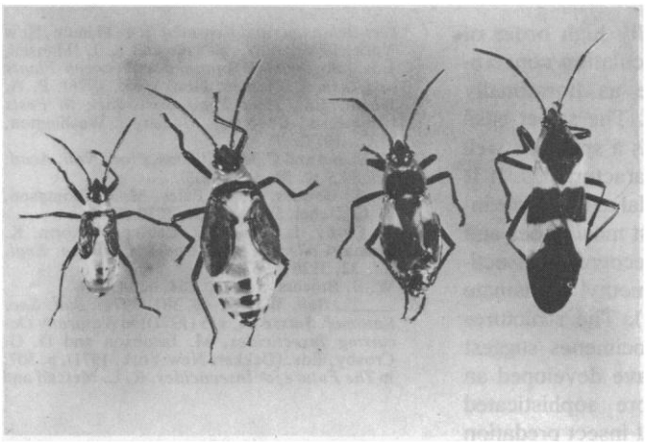

Fig. 2. Juvenilization of the milkweed bug. Treatment of a last instar nymph (left) with the juvocimenes (or juvenile hormone **1**) resulted in a dose-dependent inhibition of metamorphosis. High dosages induced the formation of perfect supernumerary sixth-stage nymphs (second from left) whereas moderate dosages allowed the formation of nymphal-adult intermediates (third from left). A normal adult is at right.

The coevolution of insects and plants has resulted in an incredible complex of chemical interactions. The use of chemicals by plants to poison, repel, or otherwise resist insect predation has been intensively studied. The presence of toxicants such as pyrethrins and nicotine or repellents such as azadirachtin in plants has been known for centuries (*11*). Only relatively recently have plant-derived chemicals with more subtle effects on insect growth, development, reproduction, and behavior been discovered. The presence of insect hormonal activity in plants was first demonstrated by Slama and Williams (*12*) when they found that paper made from or extracts of the balsam fir induced juvenilization of the linden bug *Pyrrhocoris apterus* (L.). We isolated the active constituent from the balsam fir and identified it as "juvabione" (*13*). Later Cerny *et al.* (*14*) identified a related compound "dehydro-juvabione" from a Czechoslovakian fir. Subsequently we discovered that several plant-derived insecticide synergists, including sesamin and sesamolin, possessed juvenile hormone activity against both beetles (*Tenebrio molitor*) and bugs (*Oncopeltus fasciatus*) (*2*). Combining chemical features of the natural synergists with portions of the known terpenoid structure of the natural juvenile hormones, we synthesized a group of hybrid molecules with extraordinarily high juvenile hormone activity (*2, 15*). Many other synthetic compounds have juvenile hormone activity (*16*) including some compounds containing aromatic and terpenoid features.

However, the juvocimenes are superior to most of these in activity on the milkweed bug. The basis of their high biological activity must depend upon some feature or features resident in the side chain of the juvocimenes since many compounds of indifferent activity possessing the *p*-methoxy aromatic moiety are known (*17*). The existence of an epoxide in compound **2** and its greater biological activity is not unexpected, considering the presence of a similar epoxide in the natural juvenile hormones (*18*). The characteristic branching, conjugation with the aromatic ring, and terminal olefinic conjugation of the side chain would appear to hold the secret of the superior hormonal activity. Deliberate modification of the side chain by alteration of the number, position, and geometry of the double bonds coupled with variation of the branching pattern should resolve the contributions of each structural aspect to the biological activity. Through such approaches the optimization of biologically active natural products such as the juvocimenes into safe and effective biorational pesticides may be achieved.

Although we have not tested the juvocimenes widely among a variety of in-

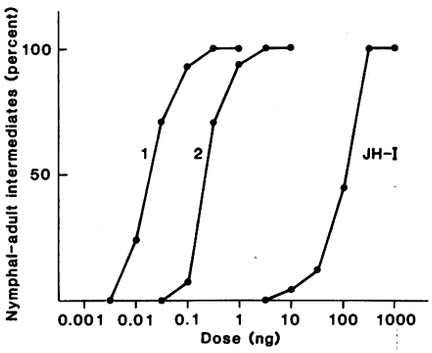

Fig. 3. Juvenile hormone activity of juvocimenes **1** and **2** in the milkweed bug juvenile hormone assay (*2*) compared with synthetic (*10*) juvenile hormone **1**. Each value represents the average modification of 30 insects.

sects, their exceptionally high order of activity encourages speculation concerning their possible role as hormonally based plant protectants. The sweet basil plant has been valued as a spice, as well as for its perfumery characteristics. It is reported to be bactericidal and to have insecticidal activity against mosquitoes and houseflies. Two of the recognized insecticidal constituents are methyl cinnamate and methylchavicol (*19*). The structures and activity of the juvocimenes suggest that sweet basil may have developed an additional and far more sophisticated chemical defense against insect predation through hormonal derangement of insect morphogenetic development.

W. S. BOWERS
R. NISHIDA

Department of Entomology, New York State Agricultural Experiment Station, Geneva 14456

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3. Mass spectra were recorded on a Hewlett-Packard 598A (GCβMSβDS) instrument at 70 eV.
4. Nuclear magnetic resonance spectra were measured on a Varian XL-100A instrument in deuterochloroform with tetramethyl silane as standard. Splitting pattern abbreviations are s, d, and t to represent singlet, doublet, and triplet, respectively. The presence of a *p*-methoxycinnamyl moiety was apparent from the following signals: δ 7.25 (2H, d, J = 9.0), 6.85 (2H, d, J = 9.0), 6.32 (1H, d, J = 15.5), 6.00 (1H, double t, J = 15.5 and 7.0), 2.24 (2H, broad t, J = 7.0 and 7.5). The double bond in conjugation with the aromatic nucleus has *trans* geometry as judged by the coupling constant J = 15.5 Hz between the olefinic protons. Two allylic methyl signals at δ 1.72 (3H, d, J = 1.5), 1.66 (3H, d, J = 1.5) coupling with an olefinic proton at δ 5.04 (1H, double septet, J = 9.5 and 1.5) suggested the presence of an isobutenyl moiety. The presence of methylbutadienyl moiety was indicated by the following signals: δ 6.38 (1H, double d, J = 17.5 and 10.5), 5.37 (1H, broad d, J = 1.3 and 9.5), 5.11 (1H, broad d, J = 17.5), 4.96 (1H, broad d, J = 10.5), 1.78 (3H, d, J = 1.3).
5. The methine proton signal was observed at δ 3.37 (1H), coupling with the adjacent two olefinic protons (each J = 9.5) and with a pair of methylene protons (J = 7.5). The relationship of the adjacent protons was verified by decoupling experiments. The geometry of the conjugated dienyl structure was determined to be *trans* through comparison of the chemical shifts with related compounds of known configuration [G. Ohloff, J. Seibe, E. Kovats, *Justus Liebigs Ann. Chem.* **675**, 83 (1964)].
6. The presence of 1.2-epoxy-2-methylpropyl moiety instead of the isobutenyl group was suggested by the presence of a pair of methyl singlets at δ 1.33 and 1.30, and a one-proton doublet at 2.71 (J = 8.0).
7. Hydrogenations were carried out in ethanol containing catalytic amounts of platinum oxide and stirred for 1 hour in a hydrogen atmosphere.
8. A Grignard reagent prepared from magnesium turnings and 1-bromo-3-(4-methoxyphenyl)-propane was reacted with 2,6-dimethyl-2-octen-4-yl acetate in the presence of cuprous chloride at −20°C in tetrahydrofuran to yield the indicated compound which on catalytic hydrogenation or epoxidation with *m*-chloroperbenzoic acid gave compounds **3** and **4**, respectively.
9. The total synthesis of both juvocimenes and several analogs have been completed (W. S. Bowers and R. Nishida, in preparation).
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Prostaglandin A Compounds as Antiviral Agents

Abstract. *Prostaglandins of the A series strongly inhibit the production of Sendai virus in African green monkey kidney cells and are able to prevent the establishment of persistent infection ("carrier" state). This action is specific for prostaglandin A and is not due to alteration in the host cell metabolism or in the virus infectivity. The possibility that this effect is mediated by interferon is discussed.*

Virus-induced tumors produce considerably more prostaglandins (PG's) than do normal cells (*1*). Prostaglandin biosynthesis is also substantially increased in Balb/c 3T3 fibroblasts transformed by simian virus 40 (*2*) and by polyoma (*3*). However, there has been little work evaluating the effect of prostaglandins on virus replication. Harbour *et al.* (*4*) demonstrated that PGE_2 (10 μg/ml) and $PGF_{2\alpha}$ (1.0 μg/ml) both increased the size of herpessimplex virus (HSV) plaques in Vero cells. PGE_2 also increased the yield of virus inoculated at low multiplicity of infection but had no effect on RNA viruses, measles virus, or Coxsackie virus B_1. In contrast, higher doses of $PGF_{2\alpha}$ (10 μg/ml) decreased virus yields. Luczak and colleagues (*5*) previously reported that PGE_2 and $PGF_{2\alpha}$ inhibited the multiplication of parainfluenza-3 virus in WISH cells and suggested that this effect could be induced by altering the rate of growth of the host cells. To evaluate the role of prostaglandins in viral replication, we have tested a spectrum of prostaglandins and prostaglandin-related compounds on the production of Sendai virus in an African green monkey kidney (AGMK) cell line (37RC) in vitro.

Stocks of Sendai virus (*6*) were prepared by allantoic inoculation of 10-day-old embryonated eggs. After 72 hours at 37°C, the allantoic fluid was harvested,

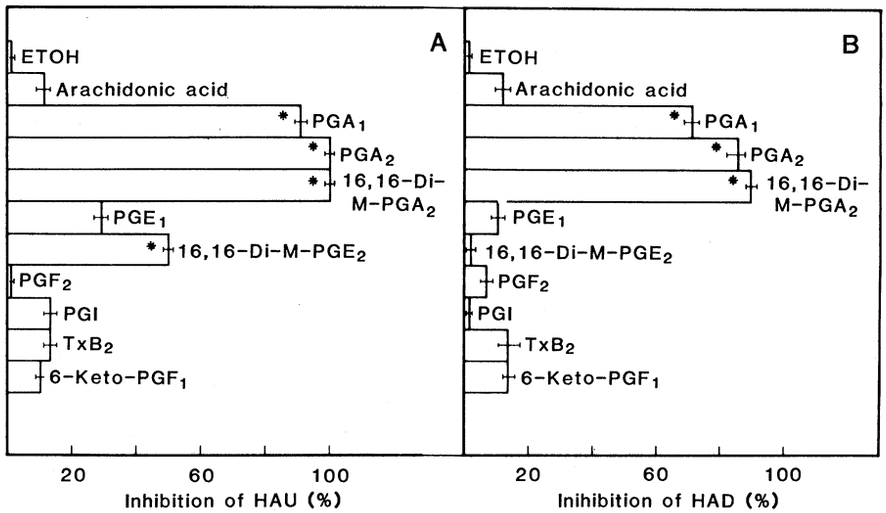

Fig. 1. Effect of different prostaglandins and prostaglandin-related compounds on Sendai virus production. Both HAU release (A) and HAD (B) are expressed as percentages of control. Each point is the mean (± standard error) of at least four cultures (*, $P < .001$). All compounds were tested at a concentration of 4 μg/ml; *ETOH*, ethanol.

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