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.

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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,

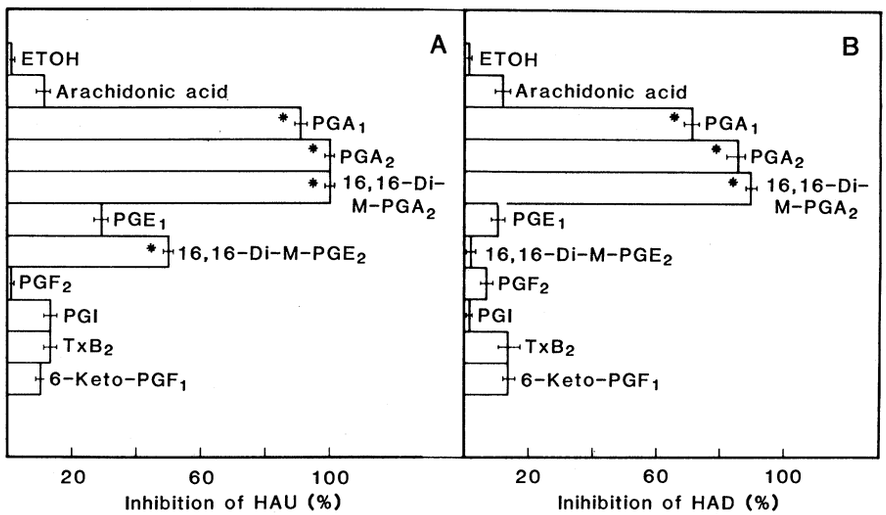

Fig. 1. Effect of different prostaglandins and prostaglandin-related compounds on Sendai virus production. Both HAU release (A) and HAD (B) are expressed as percentages of control. Each point is the mean (± standard error) of at least four cultures (*, $P < .001$). All compounds were tested at a concentration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

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clarified by centrifugation at 4000*g* for 10 minutes, and stored at −80°C. These cells, which produce interferon upon induction with Newcastle virus (*7*), were grown either in T-25 Falcon flasks or in 24-well Limbro plates in Eagle's minimal essential medium (MEM) supplemented with 5 percent fetal calf serum (Gibco) and antibiotics. They were incubated at 37°C in a humidified atmosphere containing 5 percent CO_2 and 95 percent air. Confluent monolayers of cells were washed with phosphate-buffered saline (PBS) and then infected with known amounts of Sendai virus suspension. After 1 hour of incubation at 37°C, the virus inoculum was removed and the monolayers were washed three times with PBS and then incubated with MEM (1.0 ml for Limbro wells and 10 ml for the flasks). Prostaglandin treatment was initiated after the 1-hour infection period and the medium was replaced daily. Prostaglandins and the synthetic analogs (*8*) were stored at −20°C as stock solutions in 100 percent ethanol. They were diluted to the appropriate concentrations in culture medium at the time of their use. Control media contained the identical concentration of ethanol (0.04 percent). Sendai virus titrations were performed by measuring both the hemagglutinating units (HAU) present in the medium (10.0 HAU = 10^7 virions) and hemadsorption (HAD) by infected monolayers of AGMK cells. For HAD measurements, cell monolayers were washed three times with PBS, then 0.5 ml of human red blood cells (0.1 percent, group O, Rh+) were added. After incubation at 4°C for 60 minutes the monolayers were extensively washed with PBS, and, after cell lysis occurred, the number of erythrocytes absorbed was quantitated by hemoglobin determination according to the technique of Crosby and Furth (*9*).

Figure 1 summarizes the effect of different prostaglandins and related compounds on virus production. The addition of ethanol to the medium did not produce any change. The prostaglandin precursor, arachidonic acid, did not alter either HAU or HAD, nor did PGE_2, $PGF_{2\alpha}$, prostacyclin (PGI_2), thromboxane B_2 (TxB_2), or 6-keto-$PGF_{1\alpha}$. Although 16,16-dimethyl-PGE_2-methyl ester (di-M-PGE_2) slightly inhibited HAU release, it did not alter HAD. In contrast, prostaglandins of the A series (PGA_1, PGA_2, and di-M-PGA_2) were all potent inhibitors of virus production. The inhibitory effect of PGA (Table 1) was dose dependent and was not dependent on the multiplicity of infection of the virus. At the dose used (4 μg/ml), PGA_1 was not toxic to the cells as measured both by trypan blue exclusion and by electron microscopic examination; furthermore, PGA_1 did not produce any alteration in the metabolism of control or virus-infected cells as measured by rate of cell replication and RNA and protein synthesis (*10*). Treatment of either the virus (15 minutes at 37°C) or the cells (24 or 48 hours at 37°C) before infection had no significant effect on virus infectivity or replication, respectively.

Table 1. Inhibitory effect of PGA on Sendai virus production. Each number is the mean (± standard error) of at least four different experiments. The time points represent the time after infection with Sendai virus.

Treatment	Virus production (HAU per 2×10^5 cells) at		
	24 hours	48 hours	72 hours
Control	32.0 ± 0.0	96.0 ± 0.0	48.0 ± 0.0
PGA_1 (4 μg/ml)	10.0 ± 2.0	16.0 ± 0.0	4.0 ± 0.0
Percentage inhibition*	68.8	83.3	91.7

*Compared to control.

Figure 2A shows the effect of PGA_1 (4 μg/ml) added at different times after virus infection. No difference was observed when the treatment was initiated 0, 3, or 6 hours after infection, demonstrating that PGA_1 did not act on an early event of viral replication. Moreover, PGA_1 added as late as 24 or 48 hours after infection caused comparable amounts of inhibition, indicating that the treatment was effective even on the late stages of infection. Figure 2B shows the effect of the length of treatment with PGA_1. Treatment in all cell cultures was started after the 1-hour incubation period and was stopped at different times by washing the cell monolayers three times with PBS and replacing with fresh media. Treatment for 3 hours had no significant effect on virus production; treatment for 6 hours inhibited virus production in the first 24 hours, after which time the inhibitory effect disappeared. Treatment with PGA_1 for 24 hours produced maximum inhibition at this time point, after which the inhibitory effect decreased. Finally, treatment for 48 and 72 hours resulted in a continuous maximum inhibition. These experiments demonstrated that short-term (3 to 24 hours) treatment with PGA_1 can readily be reversed, and its presence in the medium is necessary for the whole period of viral infection for the maximum effect.

Under control conditions, virus replication tended to decrease after the 48-hour peak (see Table 1). Cells that survived this first infection with egg-grown Sendai virus (about 40 percent at day 4) slowly regained normal morphology and growth potential and at 12 to 14 days af-

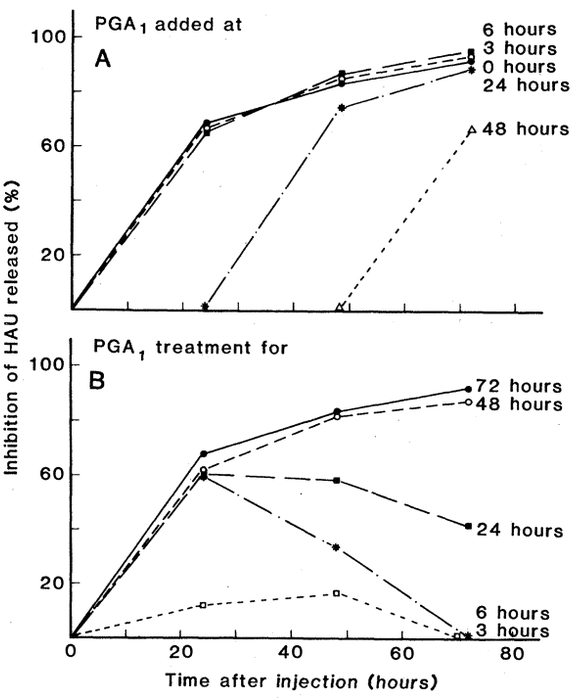

Fig. 2. Time dependence of PGA_1 treatment. (A) PGA_1 (4 μg/ml) was added at different times after infection. (B) PGA_1-treatment was suspended at different times after infection. Each point represents the mean of at least two samples. Hemagglutinin titers were identical for each pair of samples at each point (standard error of the mean is zero).

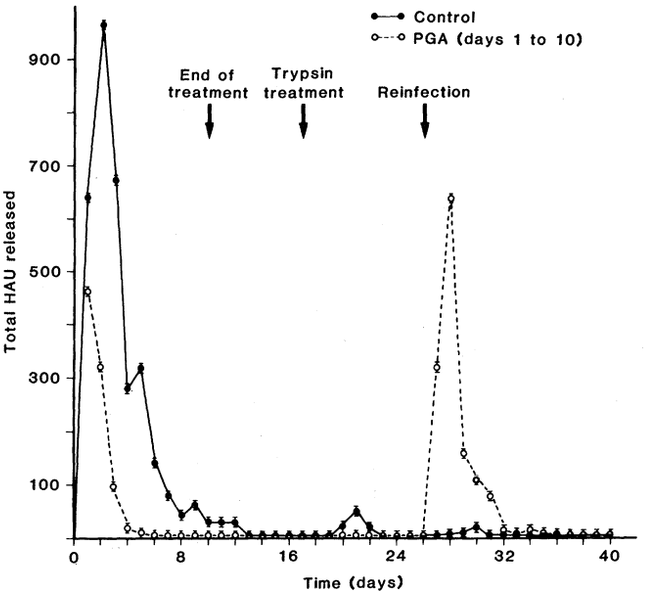

Fig. 3. Effect of long-term treatment with PGA_1 (4 μg/ml). Treatment was started after infection and continued for 10 days.

ter infection, no HAU could be detected in the medium. However, a peak of virus production was detected 48 to 72 hours after trypsinization of the cells. This indicates that the cells had become persistently infected (carriers) and were resistant to a reinfection (no increase in HAU or cell damage) by the parental egg-grown Sendai virus (*7*). As shown in Fig. 3, treatment with PGA_1 was able to prevent the establishment of a persistent infection in 37RC cells. In this experiment, PGA_1 treatment (4 μg/ml) was started soon after infection of confluent monolayers by egg-grown Sendai virus at a multiplicity of infection of 1×10^3 HAU per 10^6 cells. HAU titers were determined and medium was replaced daily. Four days after infection, the release of HAU into the medium stopped in PGA_1-treated cells, whereas control cells continued to release viruses even after 12 days. Cell morphology was similar in control and PGA_1-treated cells. After 10 days of treatment, inclusion of PGA_1 in the medium was discontinued, since cells started to detach from the plastic surface at a higher rate than was observed in control cultures. At day 17, cell cultures (control and PGA_1-treated) had regained normal morphology and were again grown to confluency. Cells were then trypsinized (0.1 percent trypsin plus 0.04 percent EDTA) for 3 minutes at 37°C and subcultured. After trypsin treatment, control cells released the usual number of HAU in the medium, while PGA_1-treated cells did not release viruses. At day 27 after the first infection, both control and PGA_1-treated cultures were exposed to a second infection by egg-grown Sendai virus (7×10^2 per 10^6 cells). Control cells did not show any change in either cell morphology or HAU release into the medium (carrier), whereas PGA_1-treated cells responded to the second infection as they had to the first infection, clearly proving that PGA_1 treatment prevented the establishment of the carrier state in these cells. Moreover, treatment of these cells 48 hours after the second infection was again effective in inhibiting virus production (data not shown).

This antiviral activity of PGA_1 is pharmacological, since the dose necessary for activity ($10^{-5}M$) is supraphysiological, cells cultured in vitro synthesize little if any PGA, and because two inhibitors of prostaglandin synthesis, indomethacin and hydrocortisone, had no effect on virus production. This action is not modulated by the production of adenosine 3′,5′-monophosphate (cyclic AMP), since addition to the medium of different doses of dibutyryl cyclic AMP did not produce any change in either HAU release or HAD (*10*). Since PGA did not alter cell metabolism and we found no alteration in viral RNA and protein synthesis, one possible mechanism is a posttranslational modification of the viral proteins by PGA_1 binding (*11*). Another possibility is that the antiviral effect is mediated by interferon. Prostaglandins and interferon have identical effects on cell replication, antibody activity, and macrophage function. Both PGA (*12*) and interferon (*13*) induce differentiation of Friend erythroleukemia cells. In addition, a relation between prostaglandins and interferon has already been established. Yaron and his colleagues (*14*) first demonstrated that inducers of interferon stimulated the synthesis of prostaglandins. Stringfellow (*15*) demonstrated the addition of PGE_2, $PGF_{2\alpha}$, and PGA_2 resulted in restoration of the interferon response after infected cells had lost their ability to respond to interferon. In a recent abstract, Chandrabose *et al.* (*16*) reported that aspirin and indomethacin, both potent inhibitors of prostaglandin synthesis, prevented the establishment of the antiviral state when interferon was added to mouse L cells in the presence of vesicular stomatitis virus. Although the mechanism is not fully understood, this antiviral property of PGA compounds may have important implications. The ability of these prostaglandins to cure the carrier state is made more relevant by the observation that Sendai virus has been implicated in the pathogenesis of multiple sclerosis (*17*).

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