the capacity of virus to enter the brain after peripheral inoculation. We showed previously that the hemagglutinin-variant viruses are extremely attenuated (*9*). We do not know whether the altered distribution of tissue injury observed in the present study is due to an altered recognition of receptors on neurons, an altered capacity of virus to spread within the central nervous system, or some other mechanism. However, the fact that the variants are altered in the region of the hemagglutinin involved in receptor binding indicates that slight changes in the viral hemagglutinin have profound effects on tissue tropism and suggest that there is an altered recognition of receptors on neurons by the viral variants. It also appears that mutations of the hemagglutinin gene occurring during the replicative cycle of a virus, perhaps even during replication within a single host, may lead to significant differences in the distribution of virus in the host. Such mutations could help explain differences in the virulence of many neurotropic viruses.

Our observations suggest that certain variant viruses might be useful as vaccines. When injected intraperitoneally, the variants did not infect the central nervous system, but did reach other organs (for example, spleen) where they could stimulate an immune response against infection with a virulent reovirus (*8–10*). The variants seem to be very stable, since we have been unable to isolate revertants from infected animals (*10*). It may therefore be possible to identify the regions of viral proteins important in virulence, select attenuated variants that are altered in their capacity to injure target tissues, and retain immunogenicity in such strains.

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A Cytokinin (Isopentenyl-Adenosyl-Mononucleotide) Linked to Ecdysone in Newly Laid Eggs of *Locusta migratoria*

Abstract. *Newly laid eggs of the insect* Locusta migratoria *contain high concentrations (50 nanomoles per gram) of an ecdysone conjugate of maternal origin; 3 milligrams of this conjugate were isolated by conventional techniques, and the structure was established by mass spectrometry and ^{1}H, ^{13}C, and ^{31}P nuclear magnetic resonance as the 22-N^{6}-(isopentenyl)-adenosine monophosphoric ester of ecdysone.*

Ovaries of vitellogenic females of the orthopteran insect *Locusta migratoria* synthesize large quantities of ecdysteroids, which accumulate inside the oocyte predominantly in conjugated form and are bound to the major yolk protein, vitellin (*1–4*). These conjugated ecdysteroids are present in the newly laid eggs and are progressively metabolized during embryonic development; their hydrolysis accounts for the presence of several peaks of free ecdysone in the eggs and embryos, two of which are monitored before the differentiation of any endocrine glands (*3, 5*). Ecdysteroid conjugates are also present in ovaries or eggs (or both) of several other insect species (*6–9*).

Two maternal conjugated ecdysteroids are predominant in newly laid eggs of *Locusta* at high concentrations (*3*); these are conjugated 2-deoxyecdysone (approximate concentration, 100 nmole/g) and conjugated ecdysone (approximate concentration, 50 nmole/g). We have identified the predominant 2-deoxyecdysone conjugate donated by the female to its offspring as the 22-adenosine monophosphoric ester of 2-deoxyecdysone (*10, 11*), and now report the isolation of the predominating maternal conjugate of ecdysone and its identification as the 22-N^{6}-(isopentenyl)-adenosine monophosphoric ester of ecdysone (Fig. 1, inset).

From about a million newly laid eggs we extracted the major yolk protein vitellin (*12, 13*); the ecdysteroid conjugates bound to vitellin (*4*) were extracted with 50 percent aqueous methanol and this extract was partitioned between hexane and 50 percent aqueous methanol. The methanol phase was subjected to reversed-phase C8 (Merck) liquid chromatography and the elution (gradient of pure water to pure methanol) of the ecdysteroid conjugates was monitored by ultraviolet absorption at 250 nm and by radioimmunoassay (*14*)—after hydrolysis by a *Helix pomatia* enzyme mixture (*2, 3*) of samples of the eluted fractions; the ecdysteroid-containing fractions were rechromatographed under the same conditions, and 3 mg of a conjugate was obtained. The hydrolysis by a *Helix pomatia* enzyme mixture of this conjugate yielded an ecdysteroid presumed to be ecdysone on the basis of thin-layer chromatography (TLC) on silica gel and reversed-phase high-performance liquid chromatography (HPLC).

The identity of ecdysone as the genin of this conjugate was ascertained by subjecting the steroid to ^{1}H nuclear magnetic resonance (NMR) analysis and to gas-liquid chromatography–mass spectrometry after treatment with trimethylchlorosilane; these results were identical to those obtained under the same conditions for reference ecdysone and were in agreement with earlier ^{1}H NMR (*15*) and mass spectrometry (*16, 17*) studies of ecdysone.

The unhydrolyzed ecdysone conjugate showed an ultraviolet absorption in methanol with a peak at 250 nm. The ^{31}P NMR spectra presented a signal at $\delta = 2$ ppm relative to phosphoric acid, indicating the presence of a phosphate group. The ^{13}C NMR spectrum of the unhydrolyzed conjugate showed a signal at $\delta =$ 80.5 ppm, which is present as a doublet ($J = 5$ Hz) in proton-noise decoupled spectra; this signal, corresponding to a carbon carrying a phosphate substituent, can be attributed to C-22 which has undergone a downfield shift of 6 ppm as a result of the presence of an electronegative phosphate group (*18*). In contrast, the signals at $\delta = 68.5$ ppm, 68.6 ppm, 73.5 ppm, and 86.5 ppm (Table 1), which are attributed in the ^{13}C NMR spectra of reference ecdysone, respectively, to car-

bons C-2, C-3, C-25, and C-14 (*19*), are not modified in the unhydrolyzed conjugate. We infer from these data that the conjugate of ecdysone is a derivative of 22-phosphoecdysone.

The ^1H NMR spectrum of the unhydrolyzed conjugate (Fig. 1) shows all the methyl signals of the genin ecdysone (at δ 0.72 : C-18 H_3; δ 0.96 : C-19 H_3; δ 0.92, doublet, J = 6.4 Hz : C-21 H_3; δ 1.20 : C-26 H_3, C-27 H_3). These methyl signals are identical to those for 22-adenosine monosphosphate of 2-deoxyecdysone (*10, 11*). The ^1H NMR spectrum of conjugated ecdysone also shows (i) two aromatic protons at δ = 7.4 ppm; (ii) two vinylic methyls at δ = 1.9 ppm; and (iii) two vinylic protons at δ = 5.9 ppm (corresponding to the proton at C-7 in ecdysone) and δ = 4.6 ppm.

Direct introduction–chemical ionization (NH_3) mass spectrometry, on either a fast heating probe (*20*) or a gold support (*21*) of the nonderivatized conjugate showed the characteristic fragments of ecdysone at (mass/charge) *m/e* 482 (MH + NH_3)$^+$; 464 (M)$^+$; 446 (M − H_2O)$^+$; 428 (M − 2 H_2O)$^+$; 410 (M − 3 H_2O)$^+$; 393 (MH − 4 H_2O)$^+$; 375 (MH − 5 H_2O)$^+$ and fragments at *m/e* 379, 336, 330, 268, 245, 212, 203, 136, 116, and 69. Some of these fragments are characteristic for N^6-(isopentenyl)-adenosine (for example, fragments at *m/e* 136, 203, 245, 268, 336) (*22*). The mass ion of the conjugated ecdysone was not observed, because of the chemical instability of this compound containing a C-O-P bond.

The data of the mass spectrometry and of ^1H NMR, ^{13}C NMR, ^{31}P NMR have led us to the working hypothesis that the conjugating moiety in the ecdysone conjugate corresponds to N^6-(isopentenyl)-adenosine monophosphate. To test this hypothesis, we have compared the mass spectrum of the ecdysone conjugate with those of reference adenosine monophosphate (sodium salt, Sigma) and N^6-(isopentenyl)-adenosine (Sigma). The spectra of all three compounds showed several characteristic peaks in common; the difference in intensities resulted from the difficulty in desorbing the ecdysone conjugate at 280°C. For an accurate comparison between the spectra of the standards and of the conjugating moiety, we therefore subjected the ecdysone conjugate to partial acid hydrolysis (HCl, *p*H 1, 60°C, 2 hours) and then purified the putative nucleoside by reversed-phase C8 liquid chromatography. This compound, which absorbs ultraviolet with a peak at 260 nm, as does standard adenosine, was subsequently analyzed by direct introduction mass spectrometry on a gold support, both with electron impact ionization and chemical (NH_3) ionization. Under these conditions, standard adenosine (Sigma) and the putative nucleoside, liberated by partial acidic hydrolysis of the ecdysone conjugate, behaved similarly. High-resolution mass spectrometry of the principal fragment of the putative nucleoside showed the exact mass of *m/e* 135.0545 ± 0.0005, which is compatible with the composition of adenine ($C_5H_5N_5$, calculated *m/e* 135.0545). These data validate the hypothesis that the conjugating moiety of the ecdysone conjugate is a derivative of adenosine. The peaks at *m/e* 203 and 336, observed in the mass spectra of the unhydrolyzed conjugate, are characteristic fragments for N^6-(isopentenyl)-adenosine (*22*); partial acidic hydrolysis of the conjugate has liberated adenosine, but not the intact N^6-(isopentenyl)-adenosine, which is explained by the cleavage of the isopentenyl group from adenosine; this reaction is also observed when reference N^6-(isopentenyl)-adenosine is subjected to the same conditions of acidic hydrolysis.

The inference that N^6-(isopentenyl)-adenosine is linked to 22-phosphoecdysone is compatible with all the mass spectral and ^1H NMR spectral data which we have given above for the unhydrolyzed conjugate; in particular, the following interpretations can be given for the fragmentations in the mass spectrum : *m/e* 69 (isopentenyl group); 136 (adenine + H^+); 203 [N^6-(isopentenyl)-adenine]; 212 (phosphoribose); 245 [N^6-(isopentenyl)-adenine + C_2H_3O]; 228 [N^6-(isopentenyl)-adenine + C_2H_4O − H_2O] 268 (adenosine); 336 [N^6-(isopentenyl)-adenosine]; 300 [N^6-(isopentenyl)-adenosine − 2 H_2O]; 348 (adenosine monophosphate); 330 (adenosine monophosphate − H_2O). In the ^1H NMR data of the unhydrolyzed conjugate (Fig. 1), the presence of N^6-(isopentenyl)-adenosine explains the signals of the aromatic protons (adenine), two vinylic methyls (in the isopentenyl group), and the vinylic proton at δ = 4.6 ppm.

The presence of the fragments at *m/e* 212, 348, and 330 (attributed, respectively, to phosphoribose, phosphoadenosine, and dehydrated phosphoadenosine) in the unhydrolyzed conjugate of ecdysone and in reference adenosine mono-

Table 1. Carbon-13 NMR data of 2-deoxyecdysone (A) and ecdysone (B) in [$^2H_5$]pyridine (internal standard, trimethylsilane); and 22-adenosine monophosphoric ester of 2-deoxyecdysone (C) and the 22-N^6-(isopentenyl)-adenosine monophosphoric ester of ecdysone (D) in ^2H_2O (internal standard, methanol); δ in parts per million from trimethylsilane; s, singlet; d, doublet; t, triplet signal.

Number of carbon atom	A	B	C	D
2	29.07 (t)	68.03 (d)	27.90 (t)	68.50 (d)
3	64.06 (d)	68.03 (d)	65.50 (d)	68.60 (d)
14	84.01 (s)	83.80 (s)	86.30 (s)	86.50 (s)
22	74.00 (d)	74.00 (d)	78.50 (d)*	80.50 (d)*
25	69.60 (s)	69.70 (s)	72.80 (s)	73.50 (s)

*In proton-noise decoupled spectra, this signal appears as a doublet.

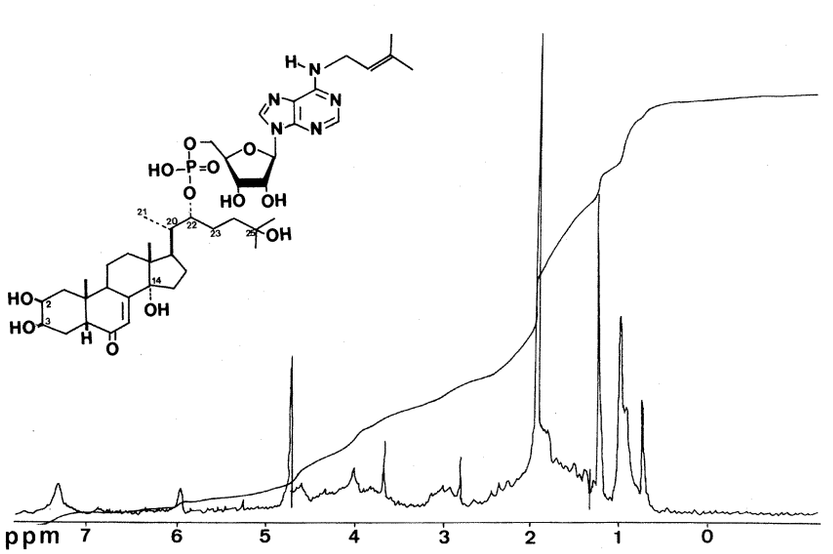

Fig. 1. ^1H NMR spectrum of the unhydrolyzed conjugate of ecdysone in D_2O; the external standard was methanol; δ in parts per million (ppm) from trimethylsilane (TMS). (Inset) 22-N^6-(isopentenyl)-adenosine monophosphate of ecdysone.

phosphate (AMP) indicate that the N^6-(isopentenyl)-adenosine is coupled to 22-phosphoecdysone at carbon C-5′.

Finally, a large signal at $\delta = 65$ ppm in ^{13}C NMR spectra of the unhydrolyzed conjugate is assigned to C-5′-O-P junction; a similar observation was reported for the covalent bond between ribose and 22-phospho-2-deoxyecdysone in the 22-adenosine monophosphoric ester of 2-deoxyecdysone (*10, 11*). Our data indicate that the ecdysone conjugate present in newly laid eggs of *Locusta* corresponds to the 22-N^6-(isopentenyl)-adenosine monophosphoric ester of ecdysone. It is not surprising that this conjugate is hydrolyzed by the enzyme mixture of *Helix pomatia*, which is known to contain phosphodiesterase together with a large variety of other enzymes. Commercial phosphodiesterase (Sigma; purified) similarly cleaves this ecdysone conjugate as it cleaves the recently identified adenosine monophosphoric ester of 2-deoxyecdysone.

The result that the ecdysone conjugate is an isopentenyl-adenosine monophosphoric ester is unexpected and of great potential interest; N^6-(isopentenyl)-adenine (i^6A) is known as a cytokinin, that is, one of a family of substances which promote cell division and exert other growth regulatory functions in plants. The nucleoside N^6-(isopentenyl)-adenosine has been found in many plants as a free nucleoside and recently in mouse cells and in human cells as a free mononucleotide; C-5′ monophosphate derivatives of a related cytokinin ribosylzeatin have also been reported (*23–25*). According to Burrows (*25*), hydrolysis of the naturally occurring cytokinin ribosides increases their biological activity up to tenfold, mainly due to the removal of the ribose yielding the more active base.

From data obtained in *Locusta* eggs (*3, 26*), we know that the maternal ecdysone conjugate is hydrolyzed progressively during embryonic development. This hydrolysis yields the free steroid hormone ecdysone either directly, or via a first hydrolytic step cleaving at the C-5′-O-P junction to the intermediate 22-phosphoecdysone, a compound that is present during embryonic development of *Locusta* (*27*) and has also been reported from eggs of the related insect species *Schistocerca* (*28*).

The fate and the role in embryonic development of the cytokinin (i^6A) liberated on the process of hydrolysis of the maternal ecdysone conjugate appears as a challenging object of investigation. In this respect, the possibility should also be left open that the isopentenyl group could be used for the construction of the carbon skeleton of juvenile hormones (*29*). This report, and the recent identification of maternal AMP-2-deoxyecdysone (*10*) in newly laid eggs of *Locusta*, unravel an extremely elaborate strategy of the maternal organism for supplying its offspring with hormonal molecules which the embryo is obviously uncapable of synthesizing de novo before advanced stages of development.

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A Glycolipid Antigen Associated with Burkitt Lymphoma Defined by a Monoclonal Antibody

Abstract. *The antigen defined by a rat monoclonal antibody directed to a Burkitt lymphoma cell line was identified as globotriaosylceramide [Galα(1 → 4)-Galβ(1 → 4)-Glcβ(1 → 1)-ceramide]. The antibody demonstrated a strict steric specificity since it did not react with globoisotriaosylceramide [Galα(1 → 3)-Galβ(1 → 4)-Glcβ(1 → 1)-ceramide], the positional isomer of the antigen associated with the Burkitt lymphoma. Chemical analysis of various Burkitt lymphoma cell lines revealed that the Burkitt lymphoma cells contained more than 100 times as much of the glycolipid antigen as was found in other human lymphoma and leukemia cell lines.*

A rat monoclonal antibody to a Burkitt lymphoma cell line (Daudi) was generated by hybridoma techniques (*1*). This antibody, designated 38-13, was found to be of the immunoglobulin M class; it defines an antigen specifically expressed on most malignant B-cell lines derived from Burkitt tumors, whether the lymphoma cells contain the Epstein-Barr virus (EBV) genome (Central-East African endemic type) or do not contain the EBV genome (European–North American type). The antigen was not detectable on EBV-positive lymphoblastoid cell lines, on normal or mitogen-activated lymphocytes, or on fresh malignant cells from patients affected with various lymphoproliferative disorders other than Burkitt lymphoma (*1, 2*). An earlier study indicated that the antigen was resistant to protease and soluble in a mixture of chloroform and methanol; it was presumed to be a glycolipid (*3*). In this report, we describe the isolation of the antigen and its chemical identification as globotriaosylceramide, which occurs in the erythrocytes of the rare blood group phenotype P^k and is called the P^k antigen (*4, 5*).

The glycolipid, which showed an in-