ble constituent of mammalian cell surfaces (*10*), it would seem more likely that *N*-acetyl-D-galactosamine participating in this interaction is provided by the cell. This possibiilty is consistent with the report that in purified PHA *N*-acetyl-D-galactosamine was not found though other sugars such as glucosamine were (*11*).

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References and Notes

1. D. A. Rigas and E. E. Osgood, *J. Biol. Chem.* **212**, 607 (1955).
2. P. C. Nowel, *Cancer Res.* **20**, 462 (1960).
3. C. B. Perera and A. M. Frumin, *Science* **151**, 821 (1966).
4. J. Borjeson, L. N. Chessin, P. Welsh, *Fed. Proc.* **25**, 306 (1966).
5. K. Heyns, G. Kiessling, W. Lindenberg, H. Paulsen, M. E. Webster, *Chem. Ber.* **92**, 2435 (1952).
6. Phytohemagglutinin was purchased as PHA-P from Difco; lot Nos. 47-97-72 and 47-13-73.
7. Minimal essential medium, H. Eagle, *Science* **130**, 432 (1959).
8. J. L. Gowans, *Brit. J. Exp. Pathol.* **38**, 67 (1957).
9. M. Tunis, *J. Immunol.* **95**, 878 (1965).
10. E. Kabat, *Blood Group Substances, Their Chemistry and Immunochemistry* (Academic Press, New York, 1956), pp. 162–183.
11. D. A. Rigas, E. A. Johnson, R. T. Jones, J. M. McDermed, V. V. Tisdale, *Fed. Proc.* **25**, 795 (1966).
12. P.M. and R.S. are career scientists, Health Research Council of the City of New York. Supported by USPHS grant CA 06657 and the American Cancer Society grant P-360.

14 June 1966

Juvenile Hormone: Identification of an Active Compound from Balsam Fir

Abstract. *A sesquiterpenoid ester with high juvenile hormone activity for* Pyrrhocoris apterus *(L.) was isolated from balsam fir,* Abies balsamea *(L.) Miller, and identified as the methyl ester of todomatuic acid.*

Extracts of various pulp woods, in particular the balsam fir, *Abies balsamea* (L.) Miller, and paper products derived therefrom, contain a factor or factors termed "paper factor" (*1*), which show strong juvenile hormone activity in the hemipteran bug, *Pyrrhocoris apterus* (L.). Thus, when last-instar nymphs were allowed to come into contact with certain paper products or were treated with lipid extractives of these products, the insect underwent one or more supernumerary molts and eventually died without becoming sexually mature. They also noted that *P. apterus* was unique in responding to the "paper factor" since two other Hemiptera, *Oncopeltus fasciatus* (Dallas) and *Rhodnius prolixus* Stal, and several Lepidoptera were unaffected. Moreover, *P. apterus* and *O. fasciatus* were reported to be little affected by highly active extracts of the cecropia silkworm which continues to be the most active source of the "natural" insect hormone. These findings have led Slama and Williams to speculate upon the possibility of chemical evolution and diversification of the juvenile hormone itself and of its target organs.

Other investigators (*2*), making independent studies of these phenomena, differ in interpreting these effects. They maintain that the "paper factor" induced a "pathological growth pattern," which resulted in abnormal or malformed adults with apparently normal reproductive organs and external genitalia, although egg production was only 10 percent of normal with few of these developing. They also declared that any juvenile hormone effect, if present, was quite small or only superficially resembled juvenilization.

Since it is always difficult to assess the biological activities of an unknown compound, particularly when it is present in an extract containing many differing molecular species, we decided that isolation of the pure material and subsequent study of its effects on *P. apterus* might clarify these inconsistencies.

Pulverized balsam fir wood was extracted in a large glass chromatographic column by perfusion with a mixture of chloroform and methanol (3:2). The solvent was removed in a vacuum and the residue was dissolved in ether; the ether solution was filtered and dried over anhydrous sodium sulfate.

A light, mobile oil with high juvenile hormone activity for *P. apterus* was isolated from this crude extract by column and preparative thin-layer chromatography over silica gel; it was 99 percent pure (or better) by gas-liquid and thin-layer chromatographic analysis (*3*). The compound was assigned the trivial name of juvabione.

Infrared analysis of juvabione in carbon disulfide showed strong absorption at 1722 cm^{-1} for an ester carbonyl group in conjugation with a double bond, and at 1645 cm^{-1} for a double bond in conjugation with an ester carbonyl. A second band at 1712 cm^{-1} indicated the presence of an additional isolated carbonyl.

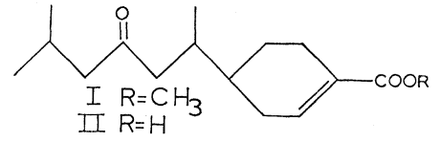
High-resolution mass spectroscopy of this ester showed a small parent ion with a molecular weight of 266.188 (*4*), a value consistent with the empirical formula $C_{16}H_{26}O_3$ (calculated, 266.187; C = 12.0000). The parent ion lost methanol which resulted in the appearance of a prominent ion at 234, a result confirming the presence of an unsaturated methyl ester (*5*).

Reduction with sodium borohydride or catalytic hydrogenation gave different products, each showing a parent ion that was two mass units higher than the original compound.

The nuclear magnetic resonance spectrum of juvabione (in deuterochloroform) showed peaks at δ (parts per million) 6.95 (1 H, olefin), δ 3.75 (3 H, methoxyl), δ 2.7 to 1.5 ($\sim$ 8 H, allylic), δ 1.5 to 1.0 ($\sim$ 5 H saturated), δ 0.88 [6 H doublet, J (spin-spin coupling constant) = 6], δ 0.86 (3 H doublet, J = 6), confirming the presence of a double bond, an isopropyl unit attached to a nonassymetric carbon atom, and a methyl group attached to an additional disubstituted carbon.

In the mass spectrum, the isopropyl fragment with mass to charge (M/e) equal to 43, a homolog M/e equal to 57 (C_4H_7) (*4*), and a related fragment at M/e 85 (C_5H_9O) were prominent and suggested the linkage $(CH_3)_2CHCH_2\dot{C}=O$.

The remainder of the molecule must contain the remaining methyl group, one ring, and the α,β-unsaturated cyclic ester. Combining these facts with the likely sesquiterpenoid nature of the material, we propose structure I.



Todomatuic acid (II), isolated from bisulfite-treated pulp wood of *Abies sachalinensis* (Schmidt) Masters, embodies all of these features (*6*). The acid, obtained on saponification of juvabione (I) crystallized from petroleum ether, gave a melting point of 57° to 59°C and a specific rotation $[\alpha]_D^{25}$ = +87° (ethanol). The physical data

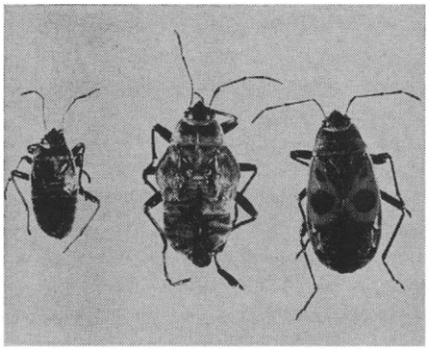


Fig. 1. The difference between the giant sixth-instar supernumerary nymph (center), normal fifth-instar nymph (left), and normal adult (right) of *P. apterus*.

are similar to those reported for todomatuic acid (*6*, *7*), m-p. 58° to 58.5°C, $[\alpha]_D^{13} = +85°$ (ethanol). Ultraviolet analysis in ethanol showed a band at 222 mμ (extinction, $\epsilon = 13{,}600$), indicative of the expected α,β-unsaturated acid. It is apparent that juvabione is the methyl ester of todomatuic acid.

Throughout purification, activity was followed by applying the material topically in 0.5 μl of acetone to newly molted last instar *P. apterus* nymphs. Supernumerary molting to giant nymphs signaled high activity (Fig. 1), while nymphal-adult intermediates were obtained with less-active fractions.

The conclusions of Carlisle *et al.* (*2*) that the balsam material produced a "pathological growth pattern" in *P. apterus* rather than juvenile hormone activity led us to compare the effects of juvabione and *trans-trans*-10,11-epoxyfarnesenic acid methyl ester on several species of Hemiptera. The latter compound is the most active synthetic juvenile and gonadotropic hormone of known structure reported to date (*8*). Table 1 shows that *trans*-10,11-epoxyfarnesenic acid methyl ester is active on all of the Hemiptera investigated; juvabione is active only on *P. apterus*, which tends to support the previously reported specificity of the balsam extract for this species. However, at higher levels (100 μg), the box elder bug [*Leptocoris trivittatus* (Say)] did molt to nymphal-adult intermediates but had such difficulty during the molt that the dorsal thoracic suture of the new cuticle ruptured and the intestines were everted through the body wall. In addition, we found that juvabione was as active as farnesol in the *Tenebrio* genitalia juvenile hormone test (*9*). In all insects responding to juvabione, the immature characters retained were like those obtained by treatment with 10,11-epoxyfarnesenic acid methyl ester. In *Tenebrio*, the juvenilizing effects of juvabione were identical with those produced by the cecropia hormone. Thus juvabione does not enjoy even ordinal specificity, despite the greater sensitivity of *P. apterus* to it. These data also indicate that the activity of the pure methyl ester of todomatuic acid is somewhat less on *P. apterus* than the activity recorded for the semipurified extracts of others (*10*); however, we were unable to detect significant activity in any other fraction from the balsam extractives. Perhaps other substances in the extract, not in themselves active, may augment the effect of juvabione, or a strain difference in test organisms may exist.

Table 1. The effect of juvabione and 10,11-epoxyfarnesenic acid methyl ester on several species of Hemiptera. The degrees of juvenilization are: O, none; N-A, nymphal-adult intermediate; and S, supernumerary sixth instar nymph. Each value represents the average modification of ten insects.

Species	Juvenilization			
	Juvabione		Epoxide	
	1 μg	5 μg	1 μg	5 μg
P. apterus	N-A	S	N-A	S
L. trivittatus	O	O	N-A	S
O. fasciatus	O	O	N-A	S
Lygaeus kalmii Stål	O	O	N-A	S

One of the most interesting aspects of our study was the discovery of a monocyclic sesquiterpene with juvenile hormone activity. Although derivatives of certain straight-chain alcohols have very high juvenile hormone activity, most mimicking compounds of known structure are derivatives of the acyclic sesquiterpenoid alcohol farnesol, which is the often proposed parent of such monocyclic sesquiterpenes as bisabolene, zingiberene, perezone, atlantone, and others, and possibly, of juvabione. Possibly the acyclic derivatives are active by virtue of their ability to cyclize within the insect, and if this is true, it follows that the natural insect juvenile hormone or hormones may also be a monocyclic sesquiterpene.

Note added in proof: We have now obtained an authentic sample of todomatuic acid through the courtesy of Dr. S. Isoe (NIH, Bethesda, Maryland). The methyl ester of this compound and juvabione are biologically equivalent, and identical by infrared spectra and gas-liquid chromatography.

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References and Notes

1. K. Slama and C. M. Williams, *Proc. Nat. Acad. Sci. U.S.* **54**, 411 (1965).
2. D. B. Carlisle, P. E. Ellis, Z. Brettschneiderova, V. J. A. Novak, *J. Endocrinol.* **35**, 211 (1966).
3. Gas-liquid chromatography was performed on a Barber Colman Model 500 instrument equipped with an all glass column 1.8 m by 6 mm (inside diameter) containing 0.75 percent SE-30 on Gas Chrom P (100 to 140 mesh), at 150°C, and with the gas flow (argon) being 15 lb/in² (1 atm). The solvent system used with both preparative and analytical thin-layer chromatography was a mixture of chloroform and methanol (97 : 3).
4. Confirmed by mass measurement on an AEI, MS-9 mass spectrometer.
5. B. Hallgren, R. Ryhage, E. Stenhagen, *Acta Chem. Scand.* **13**, 845 (1959).
6. T. Momose, *J. Pharmacol. Soc. Japan* **61**, 288 (1941).
7. R. Tuchihashi and T. Hauzawa, *J. Chem. Soc. Japan* **61**, 1041 (1940); M. Nakazaki and S. Isoe, *Bull. Chem. Soc. Japan* **36**, 1198 (1963).
8. W. S. Bowers, M. J. Thompson, E. C. Uebel, *Life Sci.* **4**, 2323 (1965).
9. W. S. Bowers and M. J. Thompson, *Science* **142**, 1469 (1963).
10. K. Slama and C. M. Williams, *Biol. Bull.* **130**, 235 (1966).
11. We thank Dr. R. I. Sailer for assistance in obtaining the culture of *Pyrrhocoris apterus* and H. Freedman, Penobscot Company, for samples of balsam fir wood.

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Proliferation of Cells in the Central Cylinder of the Reduced Mutant in Lanceolate Tomato

Abstract. *When the* reduced *phenotype in homozygous* lanceolate *tomato is cultured on a sterile nutrient medium, there is a considerable amount of cell division within the central cylinder. Such proliferation may occur in response to a stimulus furnished to the shoot by the root.*

Lanceolate tomato, a leaf-shape mutant determined by a single gene, has been described by Mathan and Jenkins (*1*) and Stettler (*2*). If the mutant allele is present in double dosage, different phenotypes are produced, one of which has a hypocotyl that lacks coty-