SYNTHESIS OF A MATERIAL WITH HIGH JUVENILE HORMONE ACTIVITY*

BY JOHN H. LAW,† CHING YUAN, AND CARROLL M. WILLIAMS

JAMES B. CONANT LABORATORY OF CHEMISTRY AND THE BIOLOGICAL LABORATORIES,
HARVARD UNIVERSITY

The juvenile hormone activity of the sesquiterpene alcohol, farnesol, and the corresponding aldehyde, farnesal,[1] has encouraged investigations of other terpenoids. In assays performed on several species of insects, Wigglesworth[2] and Karlson[3] confirmed the juvenile hormone activity, not only of farnesol, but also of two far more active compounds, farnesyl methyl ether and farnesyl diethylamine.[1] Meanwhile, Yamamoto and Jacobson[4] tested pure samples of the geometric isomers of farnesol and showed that only the *trans,trans* and the *2-cis, 6-trans* isomers were active.

Subsequently, in assays performed on pupae of the bee moth (*Galleria mellonella*) and the silkworm (*Antheraea polyphemus*), Schneiderman and Gilbert[5] found that farnesyl methyl ether and farnesyl diethylamine were approximately as active as the crude hormone extracted from males of the silk moth, *Hyalophora cecropia.*[6] Highly purified preparations of farnesol were only 15 per cent as active.   This comparison is of interest since the active principle in *cecropia* oil can be concentrated 300,000-fold without obtaining a pure substance.[7-9] Clearly, when assayed on Lepidoptera, the specific activity of the above-mentioned farnesol derivatives is far less than that of the active substance in *cecropia* oil.

In the course of our studies of farnesol derivatives, we discovered a simple procedure for synthesizing a material with remarkable juvenile hormone activity, not only for Lepidoptera, but also for nearly all orders of insects.   In view of the theoretical and practical interest of juvenile hormone, our present purpose is to describe the preparation of this synthetic material.

Synthesis from Commercial Farnesol.—Crude farnesoic acid was obtained by the oxidation of commercial farnesol, according to the procedure of Childs and Bloch.[10] The synthetic products were examined by gas-liquid chromatography[11] after conversion of the acids to methyl esters by treatment with a diethyl ether solution of diazomethane (generated from "Methyl Diazald," Aldrich Chemical Co.). *Trans,trans*-farnesoic acid and *cis,trans*-farnesoic acid accounted for less than 70 per cent of the total weight: at least four other unidentified compounds were present.

One gram of this crude farnesoic acid was dissolved in 100 ml absolute ethanol, placed in a round-bottom flask, and chilled to 0°C in an ice bath.   A stream of hydrogen chloride was passed through the solution for 5 min.   The flask was stoppered, returned to room temperature for 12 hr, and then placed on a rotary evaporator to eliminate the solvent *in vacuo* with the temperature gradually rising to 50°C. With small volumes of benzene, the material was rinsed into a separatory funnel and washed with water to remove residual traces of hydrogen chloride.   The benzene phase was collected and reduced to dryness to obtain a colorless oil of low viscosity—wt. *ca.* 1 gm.

Samples of this crude synthetic product showed high juvenile hormone activity

when dissolved in propylene glycol and injected into previously chilled pupae of the *polyphemus* silkworm. Thus, a 3+ assay[7] was obtained after injection of 25 μg into 5 gm pupae. Manifestly, one or more materials of high juvenile hormone activity are formed by the reaction of crude farnesoic acid with ethanolic hydrogen chloride.

This same procedure was repeated by exposure of crude farnesoic acid to hydrogen chloride in the presence of a homologous series of four other alcohols. When assayed on *polyphemus* pupae, the activities of the corresponding synthetic products were as follows: ethanol > methanol > n-propanol > n-pentanol. The synthetic mixture prepared in n-butanol was inactive at the highest dose tested (4 mg).

The material formed by the treatment of crude farnesoic acid with ethanolic hydrogen chloride was studied in further detail. The neutral fraction was separated by dissolving 1 gm in 100 ml ethanol. Water was added to incipient cloudiness, and the solution was neutralized with 0.1 N NaOH to a phenophthalein end-point and then partitioned between water and diethyl ether. All biological activity was recovered in the neutral (ethereal) fraction—wt. *ca.* 0.75 gm.

Attempts to Purify the Active Principle.—Thus far, the juvenile hormone activity obtained by treatment of crude farnesoic acid with ethanolic hydrogen chloride has proved refractory to extensive purification. The active material can be eluted from a column of silicic acid ("Unisil") with mixtures of benzene and pentane, but so also is the bulk of the mixture. Attempts to separate the active material by gas-liquid chromatography have not met with success, since little activity could be recovered from the columns.

In terms of its behavior in the chromatographic procedures, the active component appears to be much less polar than the juvenile hormone of *cecropia* oil, and there is no possibility that they are identical. Likewise, the active material is much less polar than the methyl ester of 10,11-epoxyfarnesoic acid—a compound which has recently been shown to have high juvenile hormone activity for *Tenebrio*.[12]

Synthesis from Pure Farnesoic Acid.—Crude farnesoic acid was prepared as described above and converted to the 2-benzyl-2-thiopseudouronium salt, according to the procedure of Bates *et al.*[13] After one recrystallization, a sample of this material (m.p. 118–122°C) was dissolved in absolute ethanol and perfused through a column of Dowex 50 (H+ form) to obtain the free acid. The rest of the crystalline salt was recrystallized three times to obtain the pure *trans,trans* salt (m.p. 133.5–134°C). The latter was converted to the free acid by passage through Dowex 50, as just described.

Samples of the two farnesoic acid preparations were converted to ethyl esters by treatment with a diethyl ether solution of diazoethane (generated from "Ethyl Diazald," Aldrich Chemical Co.). Examination of the two products by gas-liquid chromatography showed that the first contained approximately 70 per cent *trans,trans*- and 30 per cent, *cis,trans*-ethyl farnesoate. The second, more highly purified material contained at least 97 per cent *trans,trans*-ethyl farnesoate.

Both materials showed only traces of juvenile hormone activity when dissolved in propylene glycol and assayed on *polyphemus* pupae. This demonstrates that the isomeric esters of farnesoic acid have little hormonal activity.

Samples of the two preparations of farnesoic acid were treated with ethanolic hydrogen chloride, as described above. When assayed on *polyphemus* pupae, the

preparation from the mixture of *trans,trans* and *cis,trans* isomers showed detectable juvenile hormone activity at the 25-μg level; the preparation from the pure *trans,-trans* isomer showed high (3+) activity when tested at this same level.

These findings indicate that the hormonally active material is not ethyl farnesoate, but one of the lesser components formed by the reaction of *trans,trans*-farnesoic acid with ethanolic hydrogen chloride.

Tests of Cyclized Products Derived from Farnesoic Acid.—When treated with mineral acid, farnesoic acid is known to undergo a series of cyclization reactions.[14] Dr. G. Stork kindly supplied us with samples of the 2-benzyl-2-thiopseudouronium salts of α and β monocyclic farnesoic acids, as well as a dicyclofarnesoic acid (m.p. 138°C). The salts were converted to free acids by perfusion through Dowex 50 (H+ form), and the free acids were converted to ethyl esters by treatment with diazoethane. All of these esters were inert when subjected to biological assay.

Discussion.—The active material obtained by the treatment of farnesoic acid with ethanolic hydrogen chloride is neither ethyl *trans,trans*-farnesoate nor ethyl *cis,trans*-farnesoate. In terms of its chromatographic behavior, it is substantially less polar than *cecropia* juvenile hormone,[7-9] the "paper factor,"[15] or any other presently known materials with high juvenile hormone activity.

One possibility is that hydrogen chloride is added across one of the double bonds of ethyl farnesoate to give a highly active compound. In support of this hypothesis we may note that no active material was formed when sulfuric acid was substituted for hydrogen chloride in the synthetic procedure.

Summary.—In the absence of any purification, the neutral material formed in the reaction of crude or purified farnesoic acid with ethanolic hydrogen chloride is about 1000-fold more active in the *polyphemus* assay for juvenile hormone than is crude *cecropia* oil. Moreover, as documented elsewhere,[16] the synthetic material shows a far wider spectrum of activity than the *cecropia* hormone. This easily available, synthetic material is therefore of interest to scientific studies of juvenile hormone and to the practical application of the hormone in the control of noxious insects.[6]

[1] Schmialek, P., *Z. Naturforsch.*, **16b**, 461 (1961); **18b**, 513 (1963).

[2] Wigglesworth, V. B., *J. Insect Physiol.*, **7**, 73 (1961); **9**, 105 (1963).

[3] Karlson, P., *Angew. Chem.*, **2**, 175 (1963).

[4] Yamamoto, R. T., and M. Jacobson, *Nature*, **196**, 908 (1962).

[5] Schneiderman, H. A., and L. I. Gilbert, *Science*, **143**, 325 (1964).

[6] Williams, C. M., *Nature*, **178**, 212 (1956).

[7] Williams, C. M., and J. H. Law, *J. Insect Physiol.*, **11**, 569 (1965).

[8] Meyer, A., H. A. Schneiderman, and L. I. Gilbert, *Nature*, **206**, 272 (1965).

[9] Röller, H., J. S. Bjerke, and W. H. McShan, *J. Insect Physiol.*, **11**, 1185 (1965).

[10] Childs, C. R., Jr., and K. Bloch, *J. Biol. Chem.*, **237**, 62 (1962).

[11] Popjak, G., and R. H. Cornforth, *J. Chromatog.*, **4**, 214 (1960).

[12] Bowers, W. S., M. J. Thompson, and E. C. Uebel, *Life Sci.*, **4**, 2323 (1965).

[13] Bates, R. B., D. M. Gale, and B. J. Gruner, *J. Org. Chem.*, **28**, 1086 (1963).

[14] Stork, G., and A. W. Burgstahler, *J. Am. Chem. Soc.*, **77**, 5068 (1955).

[15] Sláma, K., and C. M. Williams, these Proceedings, **54**, 411 (1965).

[16] Sláma, K., and C. M. Williams, in preparation.