takes part in the maintenance of normal factor VIII concentrations in the hemophilic dog. However, they do not exclude a minor contribution by other organs, including the spleen and bone marrow. Norman, Covelli, and Sise (*2*) have reported an "experimental cure" of hemophilia by transplantation of normal spleen into hemophilic Oklahoma beagles. Their higher factor VIII concentrations may be due to the use of human substrate in the assay. Our studies were performed with canine substrate. The very low amounts of factor VIII observed in dog 2 are not subject to critical interpretation. In the case of dog 3, the bone marrow graft did not appear to influence factor VIII concentration, although long-term chimerism without secondary disease will be necessary for a definitive answer to this question.

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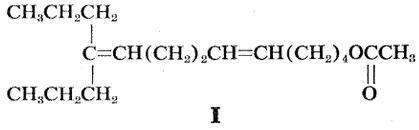
Sex Pheromone of the Pink Bollworm Moth: Biological Masking by Its Geometrical Isomer

Abstract. *A mixture of the* cis *and* trans *forms of propylure (10-propyl-trans-5,9-tridecadienyl acetate), the sex pheromone of the female pink bollworm moth, has been separated into its pure isomers by thin-layer chromatography. The* cis *isomer inhibits or masks the activity of the* trans *isomer, as little as 15 percent of the* cis *isomer being sufficient to completely nullify the activity of the* trans *isomer.*

In 1966, the sex pheromone produced by the female pink bollworm moth, *Pectinophora gossypiella* (Saunders), was isolated, identified, and synthesized (*1*). The compound, which was shown to be 10-propyl-*trans*-5,9-tridecadienyl acetate (structure I) and was designated "propylure," elicits a high degree of sexual excitement, including copulatory attempts, among caged male moths exposed to it in the laboratory.



I

Eiter *et al.* (*2*) prepared propylure by a different method and reported that it showed no activity, although the details of their biological tests were not given (*3*). They concluded that propylure is not the pink bollworm sex pheromone, despite the fact that their preparation was admittedly geometrically impure. Active propylure prepared in our laboratories was a pure single isomer (*trans*), and this prompted me to investigate their prepartion in an effort to explain the contradiction.

An explanation for the lack of activity appeared to be a possible masking effect caused by isomers admixed with propylure in the preparation of Eiter *et al.* Although these investigators discount a masking phenomenon as a factor in pheromone inhibition or inactivation (*2*), authentic cases of pheromone masking by admixed contaminants have already been reported for the sex pheromones of the gypsy moth, *Porthetria dispar* (L.) (*4*), introduced pine sawfly, *Diprion similis* (Hartig) (*5*), corn earworm, *Heliothis zea* (Boddie) (*6*), tobacco budworm, *H. virescens* (F.) (*6, 7*), American cockroach, *Periplaneta americana* (L.) (*8*), cynthia moth, *Samia cynthia* (Drury) (*9*), cabbage looper, *Trichoplusia ni* (Hübner) (*10*), and omnivorous leaf roller, *Platynota stultana* (Walsingham) (*11*). In the case of *Trichoplusia ni,* whose sex pheromone is *cis*-7-dodecenyl acetate (*12*), the agent responsible for masking is the corresponding *trans* isomer. This is likewise true for gyplure (12-acetoxy-*cis*-9-octadecen-1-ol), a synthetic sex attractant for the gypsy moth, which is completely inactivated by admixture with 20 percent of its *trans* isomer (*13*) or even smaller amounts of ricinoleyl (*cis*) alcohol (*14*).

Eiter *et al.* (*2*) reported that their preparation of propylure was a 1:1 mixture of the *cis* and *trans* isomers, and it therefore seemed highly likely that the activity of the latter was being completely inhibited by the former isomer. That this was indeed the case has now been shown by preparing propylure according to the exact procedure described by Eiter and co-workers (*2*), separating the resulting mixture into its geometrical isomers, and testing them alone and in combination for activity on male pink bollworm moths.

The propylure obtained by Eiter's procedure was a colorless liquid [b.p., 130° to 135°C at 0.1 mm-Hg; n_D^{25} 1.4610; Eiter *et al.* (*2*) reported b.p., 90° to 100°C at 0.05 mm; n_D^{20} 1.4612] whose gas-liquid chromatogram (*15*) showed one major and one minor component with retention times of 13.5 and 12.5 minutes, respectively. The minor component, comprising approximately 10 percent of the mixture, was identified as the isomeric 9-propyl-5,9-tridecadienyl acetate (*cis* and *trans*) as reported by Eiter *et al.* This contaminant was efficiently separated by slow distillation, at 0.1 mm-Hg pressure, through a spinning band column; it boiled at 129° to 131°C (n_D^{25} 1.4600), whereas the major component boiled at 134°C (n_D^{25} 1.4630).

The major component was readily separated into *cis* and *trans* forms of propylure by preparative thin-layer chromatography on silica gel impregnated with silver nitrate (*16*). With benzene-hexane (80:20) as the developing solvent, the *cis* and *trans* isomers showed R_F values of 0.31 and 0.50, respectively; propylure prepared by the method of Jones *et al.* (*1*) showed R_F 0.51 under these conditions. The

ratio of *cis* to *trans* isomer in the mixture was 40:60.

The infrared spectrum of the separated *trans*-propylure showed a medium band at 965 cm^{-1} (*trans* HC=CH) that was absent in the spectrum of the *cis* isomer, and the compound exhibited a retention time of 13.7 minutes by gas-liquid chromatography. *cis*-Propylure showed an inflexion at 740 cm^{-1} on its infrared spectrum and a chromatographic retention time of 13.2 minutes. The *cis* isomer could be converted readily into the *trans* isomer by ultraviolet irradiation of its hexane solution containing a trace of iodine.

Laboratory bioassays were conducted with the isomers and their mixtures by exposing caged male moths to the air from pipettes containing the vapors of their methylene chloride solutions (*1*, *17*). Of 100 males used in each test, approximately 75 showed sexual excitement when exposed to *trans*-propylure, whereas only 3 or 4 moths responded to *cis*-propylure, the isomeric 9-propyl-5,9-tridecadienyl acetate, or methylene chloride. Males exposed to the *trans* isomer within 15 minutes after exposure to the *cis* isomer failed to respond, but a complete response was obtained each time in consecutive exposures to the *trans* isomer alone. Vapors of *trans*-propylure containing 10 percent of the *cis* isomer elicited a response in only 15 percent of the moths, and mixtures containing at least 15 percent *cis* isomer failed to cause a response.

Thus, *cis*-propylure acts as an inhibitor or masking agent for *trans*-propylure, but the mechanism through which this occurs is not yet understood. Presumably, the vapors of the *cis* isomer act on the antennal sensory system to block temporarily the nerve endings responsible for sex pheromone detection.

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15. The chromatography was carried out on an F&M model 500 instrument equipped with a model 1609 flame-ionization attachment on stainless steel columns packed with 5 percent SE-30 on Chromosorb W (3.05 m by 0.31 cm) at 185°C; the nitrogen flow rate was 25 ml/min.
16. Adsorbosil-ADN-1, containing 10 percent calcium sulfate binder and 25 percent silver nitrate, obtained from Applied Science Laboratories, State College, Pennsylvania.
17. I thank Dr. H. M. Graham, U.S. Department of Agriculture, Brownsville, Texas, for supplying the insects used in the bioassay tests.

8 October 1968

Deoxycytidine and Radiation Response: Exceedingly High Deoxycytidine Aminohydrolase Activity in Human Liver

Abstract. *The activity of deoxycytidine aminohydrolase in the liver of various species including man was investigated. An enormously high activity was found in human liver. This fact explains the extraordinarily low level of deoxycytidine in human urine in comparison with that of other tested species. The suitability of deoxycitidinuria as a biochemical indicator of postirradiation damage in man remains, therefore, an open question.*

Pařízek *et al.* (*1*) reported the excretion of deoxycytidine in the urine of rats after irradiation with doses ranging from 10 to 600 r, in amounts proportional to the radiation damage. The suitability of that early biochemical test for radiation exposure was confirmed also for the dog, mouse, rabbit, pig, sheep, and monkey (*2*, *3*). In these studies, however, deoxycytidine was measured by the cysteine–sulfuric acid reaction described by Dishe (see *4*). Besides deoxycytidine there are also other deoxypyrimidines, as thymidine or deoxyuridine, which contribute to this reaction, but in the rat deoxycytidine remains, in normal as in irradiated animals, at least the main catabolite, which is excreted by the urine. Thus it seems that deoxycytidine is really the final biotransformation product of deoxycytidine nucleotide.

It would be of great importance if the deoxycytidinuria could be detected and made use of in irradiated human beings. But by means of the usual chemical analyses it was practically impossible to identify deoxycytidine in human urine. A very sensitive specific method was elaborated, including ion exchange chromatography and microbiological testing with the aid of *Lactobacillus acidophilus* (*5*). Arient *et al.* (*6*), using this method, have found that the excretion of deoxycytidine within 24 hours varies from undetectable amounts up to 1.5 μg, with the average about 0.5 μg per day. Only in one case of acute myeloblastic leukemia was a detectable amount of deoxycytidine found (24 μg in a 24-hour specimen) (*6*). Similarly, Berry *et al.* (*7*) could not detect any deoxycytidine in specimens collected before irradiation. After irradiation, however, a high level of deoxycytidine was found in two followed-up humans receiving therapeutic irradiation of the whole body for malignancies (*7*). Such a high level could not be confirmed by Arient *et al.* (*6*). As far as we know there are no more data about excretion of deoxycytidine in irradiated man.

There is obviously a different transformation or different degree of this transformation between rats and human beings. What is the cause of such a different level of deoxycytidine in rat and human urine? One possible explanation could be a different activity of deoxycytidine aminohydrolase, which deaminates deoxycytidine to deoxyuridine. Thus the activity of this enzyme was followed up in liver tissue of various species. Liver sample was homogenized with a 10-fold greater volume of tris buffer (*p*H 8; 0.05*M*). To 0.5 ml of homogenate, 0.1 ml of deoxycytidine solution was added (final concentration of 5m*M*), and the system was enriched with 0.5 μc of deoxycytidine-2-C^{14} (specific activity 211 mc/mmole; Amersham, England). The system was incubated for 60 minutes at 37°C and inactivated in a boiling water bath for 2 minutes; 600 μg of deoxyuridine in 0.1 ml was added as carrier and the supernatant was separated electrophoretically on Whatman No. 3 paper (formic acid 0.1*M*, 400 volts, 90 minutes). The radioactive