

A Second Compound in the Odorous Gland Liquid of the Giant Water Bug *Lethocerus indicus* (Lep. and Serv.)

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INTRODUCTION

In 1957, Butenandt and Nguyễn-Dang-Tam (1) identified the major component in the gland liquid of the abdominal reservoir of *Lethocerus indicus* (Lep. and Serv.) (*Belostoma indica* Vitalis) as the acetic acid ester of *trans*- Δ^2 -hexenol-1. The gland liquid which is considered to have a sex-attracting function in the life cycle of the insect, is called "Meng Da" by the people of Thailand, where it is used to aromatize certain dishes of their meals. Recently a sterile 5-ml sample, isolated from the glands of the insect at the Department of Science Laboratories at Bangkok, Thailand, came into our hands. Gas chromatographic analysis revealed the presence of at least nine compounds, one in such excess that it was considered to be the compound identified by the authors mentioned above.

It was thought of interest to those studying the sex-attracting activity of the liquid or its attractive odor characteristics to gather some additional information on the identity of the compound present in the second largest amount.

APPARATUS

A Becker, Delft (Holland), gas chromatograph with hot wire katharometer detector was used. In all experiments separations were obtained on a 3.60 meter, 8-mm i.d. glass column with 25% LAC-1R-296 liquid phase on 60-70 mesh Chromosorb W at 50°C. H₂ was used as the carrier gas. Compounds emerging from the column were condensed in simple glass U-tubes, cooled in liquid nitrogen.

Infrared spectrograms were obtained on a Perkin-Elmer spectrophotometer, model 13.

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For compound synthesis, standard glass laboratory equipment was used.

EXPERIMENTAL

Samples of the gland liquid were injected on the top of a gas-liquid chromatographic column. One of the resulting chromatograms is shown in Fig. 1. The two compounds represented by peaks A and B were condensed when emerging from the column and inspected by infrared (Figs. 2b and 3b). Compound A is the one considered as being identified by Butenandt and Nguyễn-Dang-Tam.

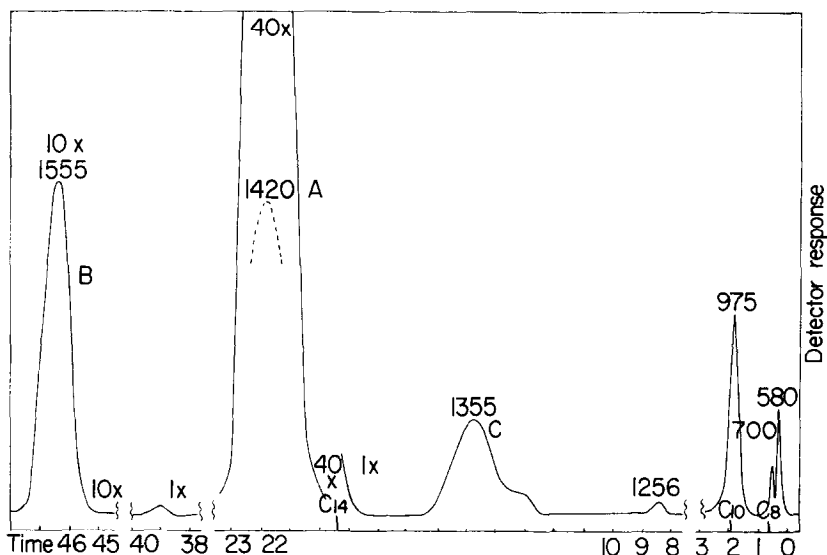


FIG. 1. Gas-liquid chromatographic analysis of volatile compounds in the gland liquid of the giant water bug. Chromatographic data in text; time is given in minutes. The figures presented with the peak maxima are the Kovats indices (K) calculated from the positions of the hydrocarbons indicated on the abscissa.

The spectrograms both showed characteristic ester and identical *trans* unsaturation in the α,β position of the alcohol portion of the ester. Of the main compound A the acid moiety could be identified by the spectrographic indices as acetic acid.

Parallel samples of the gland liquid were analyzed by thin-layer chromatography on activated silicic acid G (Merck) plates using 15% ethyl acetate in *n*-hexane as the developing solvent. After 20–30 min runs, part of the plates were sprayed with alkaline potassium permanganate, revealing two separate spots. Corresponding areas of the support

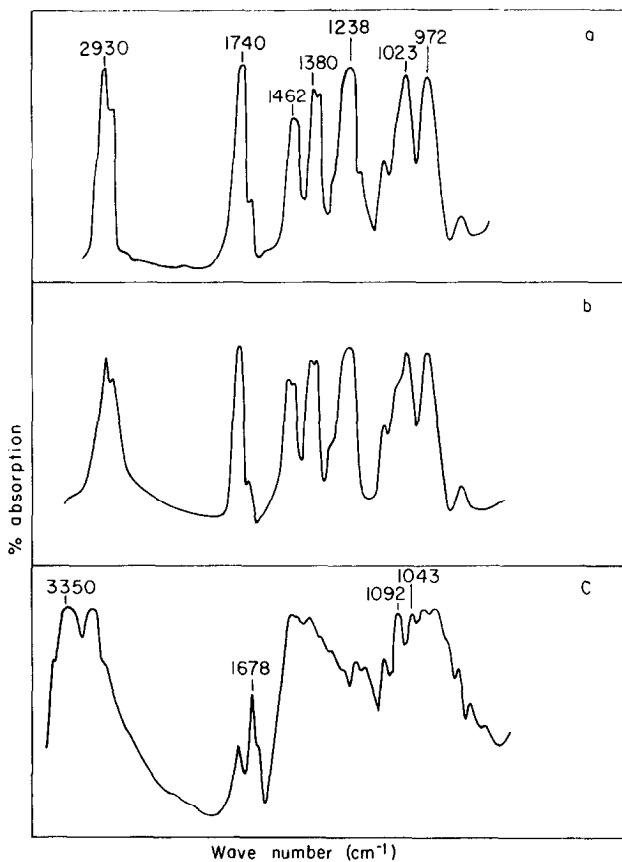


FIG. 2. Infrared spectra of the synthesized (a) and the isolated (b) *trans*- Δ^2 -hexenol-1 acetate and of the *trans*- Δ^2 -hexenol-1 (c). Wave numbers of peaks in (b) and (c) corresponding with wave numbers of peaks in (a) are not indicated.

material of the plates were extracted with ether. The ether was evaporated and the residues were taken up in CCl_4 and investigated by infrared. Spectrograms were obtained which corresponded to those of components A and B (Fig. 1) which were trapped after gas chromatographic separation.

Another portion of the same liquid sample was hydrolyzed by refluxing with an alcoholic KOH solution. The solution was acidified with H_2SO_4 and steam distilled into a morpholine solution. The salts after concentration were analyzed by paper chromatography according to Osteux, Guillaume, and Laturaze (2). Only two acid spots appeared, corresponding with acetic and butyric acid markers.

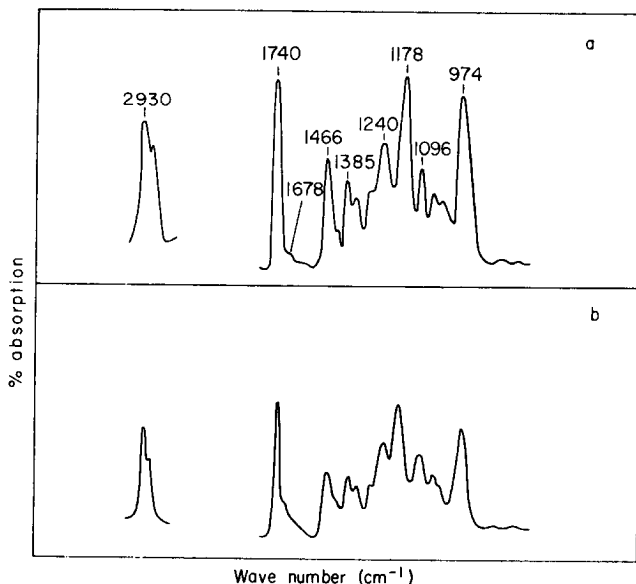


Fig. 3. Infrared spectra of the synthesized (a) and the isolated (by gas chromatography) (b) *trans*- Δ^2 -hexenol-1 butyrate. Wave numbers of peaks in (b) corresponding with wave numbers of peaks in (a) are not indicated.

Following the lead suggested by the work of Butenandt and Nguyễn-Dang-Tam, *trans*- Δ^2 -hexenol-1 was prepared for us by Chemische Fabriek "Naarden" and analyzed by gas chromatography. The material of the largest of the two main peaks was trapped at the column exit. Infrared analysis (Fig. 2c) showed the compound to be a primary *trans*-alkenol, while mass spectrometric analysis gave a molecular weight for the compound of 100 showing that the alkenol was a hexenol. Oxidative splitting of the double bond of the alcohol with alkaline potassium permanganate according to Shriner and Fuson (3) and analysis of the reaction products by paper chromatography revealed the presence of butyric acid as the main acidic product. The alcohol thus indeed proved to be the *trans*- Δ^2 -hexenol-1.

The butyric acid ester was prepared by adding butyric acid anhydride to the alcohol (molecular weight ratio 1:1) with a drop of concentrated H_2SO_4 . Analysis of the mixture by gas chromatography gave two main peaks, one of which had the same retention time as peak B of the gland liquid. The compound was condensed in a cold trap and gave an infrared spectrum identical with the one obtained by analyzing the material of peak B.

It was thus shown that the compound present in the gland liquid in the second largest amount was *trans*- Δ^2 -hexenol-1 butyrate.

When the acetic acid ester was prepared with the alcohol, a compound was isolated in the same way as with butyric acid ester, giving the same retention time and an identical infrared spectrum (Fig. 2a and b) as the one from peak A of the gland liquid. The identification of the main component by Butenandt and Nguyễn-Dang-Tam was thus confirmed.

Some information on compound C of the gland liquid was further obtained. Infrared spectrum of the trapped compound showed acetic acid ester and branched unsaturated alcohol characteristics.

DISCUSSION

It will be realized that the identification of the second compound in the insect gland liquid was eased considerably by the work of Butenandt and Nguyễn-Dang-Tam over six years ago. In our experiments the correct alcohol for the ester synthesis could be assumed right away, even when no conclusive information with respect to the chain length was obtained in our analyses by infrared spectrophotometry.

The fact that the presence of other compounds in the gland liquid was not mentioned by the former authors may well show the progress made in the field of gas chromatography over these six years. However, the gland liquid is commercially available all over the Southeast Asian countries and we have no information about the consistency of its composition; Butenandt and Nguyễn-Dang-Tam's sample may have differed in this respect from ours.

It is of interest to note that *trans*- Δ^2 -hexenal, the aldehyde corresponding to the alcohol moiety of the two esters so far identified in the gland liquid of the giant water bug, is known to occur in gland liquids of other insects (4, 5). The same aldehyde is common in green plant tissue, where it is partially responsible for the grassy odor, and is therefore often referred to as the "leaf aldehyde." The occurrence of *cis*- Δ^3 -hexenol-1, the "leaf alcohol" having a comparable odorous character, has also been reported in many plant materials.

SUMMARY

Following the work of Butenandt and Nguyễn-Dang-Tam a second compound in the gland liquid of *Lethocerus indicus* was identified as *trans*- Δ^2 -hexenol-1 butyrate.

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