HYDROCARBONS OF THE AMERICAN COCKROACH

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It is apparent from representative literature (Baker et al., 1960; Beament, 1955; Chefurka and Williams, 1951; Dennell and Malek, 1956; Gilby, 1962; Laidman and Morton, 1962; Louloudes et al., 1962; Piek, 1961; Tang et al., 1939; White et al., 1960) that there is considerable variation in the qualitative and quantitative distribution of hydrocarbons in insects. Consequently, no extrapolation can be made from the hydrocarbon content of one insect species to another. We have therefore determined the hydrocarbons of the American cockroach (Periplaneta americana (L.)) as a necessary preliminary to the use of this species for a study of hydrocarbon metabolism.

Samples of hydrocarbons were obtained from both the hemolymph and the whole carcass of the insects. Hydrocarbons from the two sources are qualitatively identical and similar quantitatively. The structures of the hydrocarbons have been determined by using material extracted from the whole roach. These compounds, once identified, were used via comparative tests to show that the hemolymph hydrocarbons are identical.

Methanol-chloroform (1:2 v/v) was used to extract the lipids from whole roaches by homogenizing them at room temperature on a high-speed blender. The filtrate of this mixture was treated with 2% (w/v) sodium chloride (ca. 1/5 the volume of the filtrate) to permit separation of the chloroform phase. The chloroform phase was dried over sodium sulfate and the solvent removed to yield a crude lipid mixture.

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Hydrocarbons were isolated from this mixture by silicic acid chromatography. An 18 g. silicic acid* (as obtained from the manufacturer) column was prepared by means of a n-hexane slurry and gravity packing. This column (2 x 12.5 cm.) was satisfactory for isolating the hydrocarbons from a 500 mg. charge of crude lipid when eluted with 80 ml. of 6% benzene in n-hexane (v/v). This fraction was identified as a hydrocarbon by its characteristic infrared spectrum and chromatographic comparison on thin layers of silica gel. A gas chromatogram of the hydrocarbons thus obtained is shown in Fig. 1. n-Pentacosane, 3-methylpentacosane, and cis,cis-6,9-heptacosadiene account for approximately 11, 20, and 65% of the total hydrocarbons, respectively.

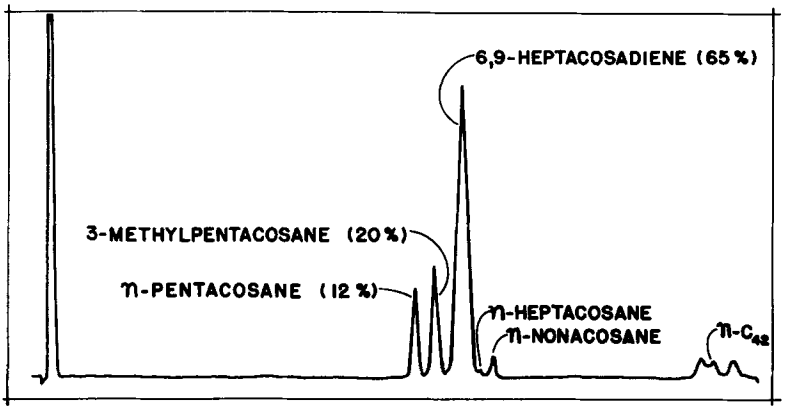

Fig. 1. GLC of hydrocarbons. 6' x 1/4" OD, 1% SE-30 on Diatoport W (60-80 mesh). Programmed - 120° - 350° C. at 5° C./min.

Identification of the material responsible for peak 1 as n-pentacosane was the result of showing it to be straight-chain, saturated, and having gas-chromatographic properties identical to known n-pentacosane. The saturated nature of the compound was established by adding a drop of bromine in CCl_4 to a n-hexane solution containing the hydrocarbon. The gas chromato-

*Unisil - Clarkson Chemical Co., Inc., Williamsport, Pa. Mention of this company does not necessarily imply an endorsement of this product by the U. S. Department of Agriculture.

gram of this material was unaltered from that of the original, indicating no bromine uptake. The straight chain was shown by the substance being adsorbed by 5-Å molecular sieve from a cyclohexane solution.

The structure of 3-methylpentacosane was arrived at by a similar approach. Saturation was established by the failure of bromine to modify its gas-chromatographic retention time. It was not adsorbed by 5-Å molecular sieve (a property which permitted its isolation from n-pentacosane) and was therefore branched or cyclic. Mass spectral data indicates that this material is a complex mixture whose principal component is 3-methylpentacosane (>95%).

6,9-Heptacosadiene was separated from the saturated hydrocarbons by application of a silver nitrate-silicic acid column (de Vries, 1962) (2 cm. dia., 18 g. of silver nitrate-treated silicic acid - 20% $AgNO_3$ w/w). The column was charged with 200 mg. of the mixed hydrocarbons and eluted with 75 ml. of n-hexane (fraction A), and 200 ml. of 30% diethyl ether in n-hexane (v/v) (fraction B). Fraction B gas chromatographed as a single peak on both SE-30 and NGS columns. Its retention time was less than n-heptacosane on SE-30 and greater on NGS, as would be expected of a straight-chain, unsaturated compound. The sample was reduced by means of Pd-charcoal and hydrogen at room temperature and atmospheric pressure. Hydrogen uptake indicated two olefinic sites, when a C_{27} system was assumed. The reduced product had the same retention time as n-heptacosane on both SE-30 and NGS columns.

The NMR spectrum of the original unsaturated compound had bands at 5.6, 2.9, and 2.2 ppm (relative to tetramethylsilane), for which a methylene-interrupted diolefin would provide suitable assignments. The proton count of these bands gave a ratio of 2.1:1.0:2.4 for the olefinic (5.6 ppm), methylene between olefins (2.9 ppm), and methylene adjacent to a single olefin (2.2 ppm) assignments, respectively. This ratio compares favorably with that of the proposed methylene-interrupted structure.

The cis,cis assignment is based upon the lack of a trans band in the 970-960 $cm.^{-1}$ region of the infrared spectrum and a J value of 6 for spin-spin coupling in the NMR spectrum.

The position of the olefinic system in the molecule has been established by producing both caproic and stearic acids by two separate oxidative cleavage procedures: (1) Ozonolysis, followed by oxidative cleavage. (2) A potassium permanganate-periodic acid oxidative cleavage procedure (Chang and Sweeley, 1962). These acids were verified as saturated acids by their infrared spectra and then converted to methyl esters for gas-chromatographic identification. Although malonic acid was not isolated, the isolation and identification of caproic and stearic acids in conjunction with the knowledge that the molecule is straight-chain C_{27} effectively positions the methylene-interrupted structure and establishes the compound as 6,9-heptacosadiene. Small amounts of butyric and valeric acids were obtained with some runs. Such quantities of these acids could result from the presence of similar but shorter chain systems, from the migration of double bond in the heptacosadiene, or both. The major products, however, were always caproic and stearic acids.

Other hydrocarbons are present in the original extract, as evinced by the gas chromatogram in Fig. 1. Although absolute identifications have not been ascertained, we have made the indicated tentative assignments on the basis of their response to hydrogenation, 5-Å molecular sieve, bromination, and comparisons with log-plot and linear presentation of gas-chromatographic data. Traces of what are probably C_{41-43} hydrocarbons are also present, but no characterization has been made.

The hemolymph used for total hydrocarbon determination was obtained by direct sampling from the thoracic cavity through an opening 0.5 cm. in diameter in the dorsal cuticle. Prior to the operation the area was well rinsed with n-hexane to minimize possible contamination from the cuticular wax. The fluid obtained was transferred directly to a silica-gel thin-layer chromatography plate and developed with n-hexane. Two hydrocarbon spots were clearly visible (Rf approx. 0.85 and 0.79). These areas were removed and eluted to obtain the hydrocarbons for gas chromatography. The lead spot gave gas chromatographic peaks at positions identical to those of the n-pentacosane and 3-methylpenta-

cosane. The trailing spot chromatographed as 6,9-heptacosadiene, and had an IR spectrum indistinguishable from the corresponding whole-insect hydrocarbon.

The determination of hemolymph hydrocarbon content was made by transferring hemolymph directly to a weighed tube containing approximately 0.2 g. of silicic acid and reweighing on a microbalance. This material was then placed on top of 4.5 g. silicic acid chromatography column (1.0 x 10 cm.) and eluted with 40 ml. of 6% benzene in n-hexane (v/v). The eluate was collected as a single fraction, reduced in volume with a rotary evaporator, transferred to a tared vial, and the remaining solvent removed with a stream of nitrogen. The vial was reweighed to determine the weight of hydrocarbons. Hemolymph hydrocarbons represent 0.35% of the total in males and 0.68% in females.

The hydrocarbon fraction of the American cockroach is the simplest of any insect yet reported, and there is more detailed information about the individual hydrocarbons of this insect than is available from current knowledge of other insects. The American cockroach is thus well suited for investigations of the metabolism of insect hydrocarbons. Studies on the biochemistry of the hydrocarbons of this insect are currently being undertaken in this laboratory.

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