TWO NEW TRYPtopHANE METABOLITES OF THE AMERICAN COCKROACH

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Abstract—In the course of attempts to isolate and characterize the 'nesting' or aggregation pheromone of the American cockroach, Periplaneta americana, two metabolites of tryptophane have been identified. These are oxindole and hydrocarbostyril. That these were indeed tryptophane metabolites was demonstrated by administering radiolabelled tryptophane to cockroaches following tetracycline treatment and isolating the radioactive metabolites.

INTRODUCTION

LARVAE of the American cockroach, Periplaneta americana, are attracted to hiding places populated by adult cockroaches by the presence of an aggregation pheromone in cockroach excreta (BLOCK and BELL, 1974). To study aspects of the chemical triggering of behaviour patterns, it is of interest to isolate and characterize the pheromone responsible for aggregation.

MULLINS and COCHRAN (1973) have examined the excreta of P. americana and find that ammonia, amino nitrogen, unidentified water-soluble products, and some water-insoluble materials and three metabolites of tryptophane are among the nitrogenous wastes produced. We reasoned that the aggregation pheromone might well be in the water-insoluble fraction of the faeces and have examined organic solvent extracts of the excreta for the pheromone. It is in these that two new metabolites of tryptophane are found.

MATERIALS AND METHODS

A flow chart of the extraction procedure for an active fraction producing aggregation (as determined by bioassay, BLOCK and BELL, 1974) is presented in Fig. 1. The active fraction was further purified by preparative thin-layer chromatography (Silica gel GF254, Brinkman Instruments Inc., 2 mm) using 15% acetone in methylene chloride as the developing solvent. Visualization of the plates under u.v. light (254 nm), recovery of the adsorbing substances, and bioassay provided an active fraction at \( R_f \) 0-3 to 0-4.

Active fractions from several plates were combined and sublimed (60°C at \( 1 \times 10^{-5} \) Torr). The sublimate was collected (2.5 mg) and subjected to mass spectrometry (Varian-Atlas CH 5, by solid inlet), i.r. spectrometry (Beckman
Faeces

↓

Ether extracts

↓

Evaporate

↓

Residue

Partition hexane

(inactive)

Methanol : 5% water

↓

Evaporate

↓

Residue

Ether

H₂O (inactive)

↓

Chromatography*  
(preparative TLC)

Fig. 1. Flow scheme for the isolation of aggregation stimulating fraction.

*Active fraction = \( R_f \) 0.3 to 0.4 in acetone–dichloromethane (1 : 5).

IR 10), u.v. spectrophotometry (Cary 14), and proton magnetic spectrometry (Varian HR 220). (We wish to thank Professor E. Wenkert, Indiana University, Bloomington, Indiana, for providing the NMR data.) Gas chromatography of the sublimate was preformed on a 6 ft. x ½ in. o.d., 10% Ucon W98 on chromosorb G, stainless steel column with a Hewlett-Packard 5750 B gas chromatograph temperature programmed from 200 to 250°C at 4°C/min and a flow rate of 40 ml of helium/min.

Chemical procedures

Authentic hydrocarbostyril and oxindole were prepared by the method of Blout and Silverman (1944). The products of the catalytic reductions were recrystallized to constant melting points from water.
Carbon-14 ring labelled tryptophane was obtained from Schwartz–Mann (sp. act. 29 MCl/m-mole/50 μCi/ml). Tetracycline (Lederle) was a gift from the School of Pharmacy, University of Kansas.

**Animal rearing and feeding study**

Experimental cockroaches (both male and female) were given ample Purina laboratory chow but denied water for 3 days, and then provided water containing 0.5 (w/v) of tetracycline. This procedure was repeated four times. Repetition of this procedure with substitution of the water containing antibiotic with water containing radiolabelled tryptophane (a total of 15 μCi) was accomplished over a 9 day period. The animals were maintained on lab chow with free access to tap water for 1 week more. The faeces was collected and subjected to the extraction procedure (Fig. 1). One milligramme and four milligrammes each of oxindole and hydrocarbostyril were added to the extract and the chromatographic and sublimation purifications carried out. The resultant sublimate was recrystallized to constant specific activity (Table 1).

### Table 1—Specific activity of isolated metabolites

<table>
<thead>
<tr>
<th>Substance</th>
<th>Specific activity (μCi/μmole) (×10^-4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sublimate</td>
<td>0.94</td>
</tr>
<tr>
<td>First recrystallization</td>
<td>1.73</td>
</tr>
<tr>
<td>Second recrystallization</td>
<td>1.78</td>
</tr>
<tr>
<td>Third recrystallization</td>
<td>1.70</td>
</tr>
</tbody>
</table>

**RESULTS AND DISCUSSION**

Gas chromatography of the fraction isolated following sublimation demonstrated the presence of two materials, one of shorter retention time in about one-quarter the concentration of the longer retention time substance. Infrared spectroscopy indicated the presence of a carbonyl absorption (broad 1705–1690 cm⁻¹) and an aromatic ring (1605 cm⁻¹). The position of the carbonyl absorption suggested an amide function. The u.v. spectrum of the material contained a peak of absorption at 247 nm. The nuclear magnetic resonance spectrum was quite instructive. At 9.95 δ (ppm from tetramethylsilane internal standard) was a broad singlet of approximately one proton, at 6.6 to 7.5 δ was a complex multiplet which represented four protons, at 3.54 δ was a sharp singlet of approximately one-half of a proton, and at 3.02 and 2.64 δ were two triplets (J = 7 Hz) which integrated for just less than two protons each. These observations suggested a material containing a disubstituted aromatic ring with the presence of only a two or three carbon side chain. Finally the mass spectral data allowed us to deduce a possible structure(s). The apparent molecular ion was at m/e = 147. High
resolution analysis indicated that it had an empirical formula of $C_9H_9NO$. A significant peak at $m/e = 133$ of empirical formula $C_9H_9NO$ was also present. Since a loss of $m/e = 14$ is a fairly uncommon fragmentation pattern, we surmised that the substance was actually a mixture of homologues. This is also borne out by the gas chromatographic result. Additional strong peaks at $m/e$ of 118, 119; 104, 105; 77, 78 strongly support the presence of the substituted aromatic ring. The structure best fitting the above data was a pair of homologous cyclic amides—oxindole and hydrocarbostyril. That these were indeed our unknowns was shown by comparison of the authentic substances with the isolated material. The physical and spectral properties of the metabolites were completely identical with those of a 1:4 mixture of oxindole and hydrocarbostyril.

Bioassay of this synthetic mixture for aggregation pheromone activity proved to be negative. Re-examination of freshly sublimed isolates also proved to be negative while material trapped in the vacuum pump cold traps during the sublimation was very active. The actual pheromone must be somewhat more volatile than these two metabolites.

To determine the source of these unusual substance, we conducted a series of feeding experiments. Since our metabolites were reasoned to be tryptophane derivatives, radiolabelled tryptophane was fed to bacteria-free cockroaches. Re-isolation of the metabolites from the faeces of these cockroaches did produce radiolabelled oxindole and hydrocarbostyril (Table 1).

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REFERENCES

