Chemical Studies on the Sex Pheromone of *Tenebrio molitor* (Coleoptera: Tenebrionidae)[1]

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Preliminary studies on the isolation and chemical characteristics of the sex pheromone of the female yellow mealworm, *Tenebrio molitor* L. (Valentine 1931, Tschinkel et al. 1967), have been carried out. The pheromone consists of at least 2 synergistically acting compounds, one extractable in pentane (fraction A), the other in more polar solvents (fraction B). A 1:1 mixture (v/v) of the 2 fractions showed the same level of activity in the bioassay (Tschinkel et al. 1967) as the control extracts in tetrahydrofuran, whereas individually each fraction had only low activity (Table 1). All subsequent tests were thus made after recombination of the sample with an equivalent amount of the complementary fraction.

Fraction A was unstable above ca. 60°C and was not recovered after exposure to chromatography on alumina. Fraction B, on the other hand, was stable at least to 80°C. Treatment of fraction B with acetyl chloride and other hydroxyl reagents gave no decrease in activity, and it was concluded that the compound probably contained no free hydroxyl group. The sex pheromone of fraction B was extractable into aqueous base (0.1N NaOH; 2% Na_2CO_3), and reextractable into fresh ether (fraction B-1) after acidification of the aqueous phase. Activity of this fraction (69±3.8% of test males responded positively) was lost upon methylation with diazomethane (21±5.1%) and regained after saponification by stirring the ether phase over 1 N KOH for 30 min (59±4.4%). All test solutions contained 1 FE of fraction B-1 and 1 of fraction A in 1 ml of solvent. Activity was lost upon treatment with lithium aluminum hydride and sodium borohydride. It is hypothesized that the activity of fraction B results from an acid. The very rapid saponification of the methyl esters and the loss of activity upon treatment with dilute bromine solutions suggest that an unsaturation may also be present.

Column chromatography on silica gel and florisil of both the free acids and the methyl esters gave poor separation as did thin layer chromatography on silica gel, gel filtration, and ion-exchange chromatography in tetrahydrofuran:water (3:1 v/v). Silica gel TLC, while resulting in discrete spots upon treatment of the plates with iodine vapor, did not separate the sex pheromone activity of fraction B-1. Though vacuum distillation was partially successful, activity could be recovered from gas-liquid chromatography of the methyl esters in only 1 out of 4 trials. No activity was recovered from the GLC of the free acids.

Table 1.—Response of male *T. molitor* to pentane and tetrahydrofuran extracts, alone and combined, of female beetles.

Procedure	Activity (% responding positively)[a] Alone[b] ±SE	Combined[c] ±SE
Females (2/ml) extracted 4 hr in pentane (Fraction A)	5±2.7	
Followed by 12 hr in tetrahydrofuran (Fraction B)	15±4.3	61±3.6
Control: females (2/ml) extracted 4 hr in tetrahydrofuran	55	
Followed by 12 hr in pentane	5	40

[a] A positive response was scored when the male attempted to copulate with a glass rod treated with the test extract.
[b] Alone: ½ ml of the fraction and ½ ml of solvent.
[c] Combined: ½ ml of fraction A and ½ ml of fraction B.

Great difficulty was encountered in bioassaying for 1 of 2 or more synergists. As long as the compounds were present in their natural ratio, the bioassay is accurate and reproducible. However, deviation from this ratio caused great variability and loss of sensitivity, making interpretation of the data difficult. Since fraction B had low activity independent of fraction A, it is possible that the bioassay difficulties can be overcome by testing fraction B by itself in high concentrations.

Happ and Wheeler (1969) recently reported some purification of the female sex attractant of *T. molitor* by column chromatography. Their bioassay relied on *attraction* as the criterion for sex pheromone activity, while I have depended on *attempted copulation*. At present it seems possible that these 2 behavior patterns are released by different compounds, for there was no report of whether Happ and Wheeler's compound released copulation in addition to being attractive to males. The column chromatographic behavior reported by Happ and Wheeler differs so greatly from that of fraction B of my study that it seems likely that 2 compounds are involved. The possibility still remains that fraction A is identical with Happ and Wheeler's compound.

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