

## Isolation of a Substance that suppresses Feeding in Locusts

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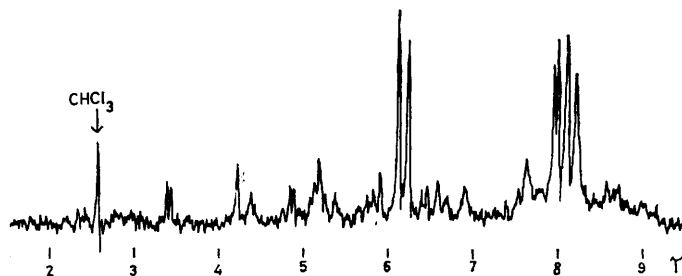
WE report the isolation of a substance from the seeds of *Melia azadirachta* (*M. indica* or *Azadirachta indica* A. Juss) which inhibits feeding in the desert locust (*Schistocerca gregaria*). We are prompted to record our preliminary investigations by the report that meliantriol from the fresh fruit of *Melia azedarach* and the oil of *M. azadirachta* is a feeding repellent for the desert locust.<sup>1</sup> The inhibiting substance, which we call azadirachtin, is not related to meliantriol and is of notably high activity as a feeding repellent.

Feeding tests were carried out by impregnating filter paper (5.5 cm. discs of Whatman No. 1) with methanol solutions of fractions from the seeds at various dilutions. The papers were momentarily dipped in the solution, allowed to dry, sprayed with 0.25M-sucrose and dried again. These test

papers were presented to separate groups of mid 5th instar hoppers of *S. gregaria* which had been starved for about 24 hr.

The seeds of *M. azadirachta* were macerated with ethanol in the cold, and the ethanol extract subjected to successive solvent partitions followed by alumina chromatography. Fractionation was monitored by feeding tests and thin-layer chromatography. It was possible to correlate all activity with a narrow range of  $R_f$  values in t.l.c. Further chromatography gave colourless amorphous material, which completely inhibited the feeding response at a concentration of 5 mg./l.

Further purification by multiple-elution preparative layer chromatography and crystallization from carbon tetrachloride gave azadirachtin as a microcrystalline powder, m.p. 155—158°,  $[\alpha]_D$



FIGURE

$-53^{\circ}$  [ $c$  0.5 ( $\text{CHCl}_3$ )],  $M^+$  642.222 (calc. for  $\text{C}_{29}\text{H}_{38}\text{O}_{16}$ , 642.216)  $\lambda_{\text{max}}$  217  $m\mu$  (EtOH)  $\log \epsilon$  3.9,  $\nu_{\text{max}}$  3460, 1745, 1720, 1655, 1620  $\text{cm}^{-1}$  ( $\text{CCl}_4$ ). The n.m.r. spectrum (Figure) showed a complex series of absorptions between  $\tau$  3.3 and  $\tau$  8.8, there were no strong absorptions between  $\tau$  8.6 and  $\tau$  10.0 corresponding to the angular methyl groups of a triterpenoid, nor were there any absorptions in either the n.m.r. or infrared spectra characteristic of the furan ring present in many of the triterpenoids found in this and other members of the family *Meliaceae*. The absence of absorption in the  $\tau$  9.3—8.6 region is taken as

clear indication that azadirachtin is not related to meliantriol. Azadirachtin has 100% anti-feeding activity at a concentration of 70  $\mu\text{g./l.}$  In the absence of details from Lavie *et al.*,<sup>1</sup> we tentatively calculate this to be equivalent to 1  $\text{ng./cm.}^2$  by their method.

The elucidation of the structure of azadirachtin is proceeding and will be reported later.

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<sup>1</sup> D. Lavie, M. K. Jain, and S. R. Shpan-Gabrielith, *Chem. Comm.*, 1967, 910.