II. CARBOHYDRATES  

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ABSTRACT  
Honeydew of the lac insect, *Kerria lacca*, is rich in sugars. By means of paper partition chromatography four carbohydrates, viz., glucose, fructose, sucrose and an unidentified oligosaccharide have been detected in the honeydew of mature females. The sap of a host-plant, *Mogania macrophylla*, was also analysed and found to contain only first three of them. Probable identification of the unidentified compound has been discussed.  

INTRODUCTION  
The honeydew of aphids and coccids contains much unabsorbed organic matter, e.g., carbohydrates, amino acids and amides (Rockstein, 1964; Wigglesworth, 1965). In freshly excreted honeydew water soluble carbohydrates account for more than 80% of the total weight, the remaining 20% being water, nitrogen containing compounds and traces of other material (Ewart and Metcalf, 1956). Among the nitrogenous constituents uric acid is reportedly most common in the excreta of insects.  

Little is known about the honeydew of the lac insect, *Kerria lacca* (Kerr). The amino acids and amides present in the honeydew of mature females have been reported in the Part I of the present study (Srivastava and Varshney, 1966). It was not possible to detect uric acid in the lac insect honeydew. Mittler (1958) also failed to find evidence of uric acid in case of *Tuberolachnus salignus*. However, the honeydew of lac insect is quite rich in sugars, as evident from the large number of ants which are attracted to it, as well as by the growth of a sooty black fungus on the surfaces of leaves and shoots where honeydew droplets fell.  

The present study was carried out initially at the Indian Lac Research Institute, Ranchi, and later continued at the Deptt. of Zoology, Patna University. It forms a part of the thesis of the author, approved for the Ph. D. degree of the Patna University (Varshney, 1972).  

MATERIAL AND METHODS  
The composition of honeydew was analysed by the paper partition chromatography. Fresh honeydew droplets from mature lac insect females growing on experimental host-plants, particularly *Mogania macrophylla*, were used. Fresh droplets were collected by means of a fine micropipette and these were deposited directly on the chromatographic filter papers. Chromatograms were developed unidimensionally as well as two—dimensionally. The
first development was made in phenol-water (80 : 20 v/v) and the second in n-butanol-ethanol-acetone-water (5: 4: 3: 2 v/v). Some other solvent systems were also tried for the second development, viz., n-butanol-acetic acid-water (4: 1: 5 v/v, upper layer), and ethyl acetate-acetic acid-water (4: 1: 5 v/v, organic phase). The dried chromatograms were treated with benzidine-trichloroacetic acid reagent by dipping technique of Harris and MacWilliam (1954). The silver nitrate method of Trevalyan et al. (1950) was also tried, but as it was observed to make spots heavier, it was abandoned. The carbohydrates were identified by comparing the \( R_f \) with standard chromatograms, which were

Fig. 1. Circular chromatogram showing some pure carbohydrates, and the carbohydrates separated from the honeydew of *Kerria lacca* and the sap of host-plant *Moghania macrophylla*: 1. Honeydew, 2. sucrose, 3. trehalose, 4. arabinose, 5. melibiose, 6. maltose, 7. sap of host-plant, 8. raffinose, 9. lactose, 10. melizitose, 11. fructose 12. glucose.
prepared from known pure carbohydrates developed in exactly similar conditions.

Circular chromatography was also attempted for analysis. Such chromatograms were spotted with the honeydew of lac insect female, sap of the *Moghania macrophylla* host-plant, as well as a number of pure carbohydrates side by side, and developed horizontally in n-butanol-ethanol-acetone-water (5:4:3:2 v/v) solvent system. The spots were revealed by the benzidine-TCA reagent.

**RESULTS AND DISCUSSION**

The honeydew of lac insect, *K. lacca*, is a clear watery fluid in the fresh state. On getting dry, however, its drops become white or yellowish-white due to presence of heavy quantity of sugars. Four carbohydrates have been detected in the present study. These are glucose, fructose, sucrose and an unidentified oligosaccharide compound. The sap of the host-plant *Moghania macrophylla* was found to contain only first three of them, the fourth sugar was absent (Fig. 1).

This fourth unidentified compound invites attention. It appeared on the chromatograms below the spot of sucrose and its Rf value suggested it to be an oligosaccharide. The compound apparently comes closer to raffinose and melibiose, as shown in Fig. 2. It is well known that the heavier molecular weight of a compound lowers its Rf value. By comparing the unidentified carbohydrate compound with other known pure carbohydrates, the possibility of the former being a monosaccharide or disaccharide does not exist. It may, hence, most probably be a trisaccharide.

Many other carbohydrates have been recorded from the honeydew of other coccids and aphids. Gray and Fraenkel (1953) discovered a trisaccharide in the honeydew of aphid, *Aphis spiraeola* and two coccids, *Pulvinaria vitis* and *Pseudococcus citri*. Bacon and Dickinson (1957) have showed that a trisaccharide, melizitose, present in the honeydew of *Eucallipterus tiliae* was an insect product and produced due to the enzymatic action on sucrose within the insect body. In the honeydew of another coccid, *Icerya*...
the presence of trisaccharide melizitose has been reported along with an enzyme which is said to be capable of synthesizing it from sucrose present in that insect (Ewart and Metcalf, 1956). The unidentified carbohydrate of the honeydew of lac insect, reported in the present study, is evidently produced during the metabolic activity within the insect body, as it is absent in the sap of the host-plant (Fig. 1). This carbohydrate might have been produced due to the action of an enzyme, perhaps invertase, on other carbohydrates present in the gut of the lac insect. Such a possibility is expected since it is now established that invertase is capable of forming oligosaccharides due to the transglucosylase activity, which has been reported in many plant sucking insects (Duspiva, 1953, 1954; Saxena and Bhatnagar, 1961; Srivastava and Auclair, 1962).

It seems that like other coccids and aphids, the lac insect also takes amino-acids and carbohydrates from the host-plant in excess of its requirement and thus passes this excess in the honeydew. The significance of this wasteful method of feeding is generally supposed to lie in a deficiency of some compound in the sap of host-plant, which is unknown as yet and deserves investigation.

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REFERENCES


