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The Composition of the Honeydew Excreted by Australian Coccids of the Genus *Ceroplastes*

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The coccids included in the genus *Ceroplastes* are popularly known as 'wax scales' from the large amount of waxy secretion covering the adult female. Froggatt (1921) recognized three species as occurring in Australia, namely, *C. ceriferus* And., *C. rubens* Marshall and *C. rusci* L. A fourth species, *C. floridensis* Comst., is recorded in the catalogue of Fernald (1903) as occurring in Australia, and Froggatt remarks that if this is the case it is a rare and comparatively unknown coccid. Later (Zeck, 1932) it was shown that another species, *C. destructor* Newstead, is of wide distribution and that it was formerly confused with *C. ceriferus*. All these coccids are considered to be introduced species, although *C. rubens* was originally described from Australia (Froggatt, 1921).

These wax scales are pests and occur in tropical and subtropical regions. *C. ceriferus* (Indian white wax scale), although widely distributed, only infests a few garden plants and native shrubs, whereas *C. destructor* (African white wax scale) and *C. rubens* (pink or red wax scale) are pests of citrus trees and they also attack many other introduced and native trees and shrubs. *C. rusci* (a white wax scale), similar to *C. ceriferus* though smaller, has been reported as occurring on melaleucas growing on the far north coast of New South Wales and on the Queensland coast. Honeydew is produced by the insects, often in large quantities, and sooty moulds, which develop in association, may cover the leaves, stems and fruit upon which the secretion falls.

Little is known about the composition of the honeydew secreted by coccids, and nothing appears to have been published on the honeydew produced by the genus *Ceroplastes*, although Fraser (1937) has reported briefly on some results obtained with

material supplied from the early stages of this work. The coccid *Trabutina mannifera* feeding on tamarisk in Sinai produces a secretion (manna) which contains 55% sucrose, 25% invert sugar and 19.3% dextrin (Weber, 1930). Gontarski (1940) records the composition of the honeydew secreted by *Physokermes piceae* Schr. (*Lecanium hemicryphum*) as containing no fructose, 11.4% water, 28.1% dextrin, 14.3% glucose and 35.8% sucrose, and that secreted by *L. quercus* (L.) Burmeister as containing 16.3% water, 11.1% dextrin, 43.7% glucose and 39.9% sucrose. More information is available on the composition of the honeydew produced by another hemipterous family of the suborder Homoptera, namely, the family Aphididae (Michel, 1942; Molliard, 1931; Mawson, 1948). However, except for the paper by Molliard, no reference is made to the presence of sugar alcohols. Molliard reports that the excretion of *Aphis euonymi* Fabr. feeding on *Euonymus europaeus* L. contains quantities of dulcitol which are apparently obtained from the leaves of the host and pass through the insect unchanged; sugars are also present.

EXPERIMENTAL

Separation of the honeydew

From Ceroplastes destructor. A mixture of insects, wax and honeydew was collected from the branches of infested lemon trees and most of the honeydew separated by pressing and draining. The remainder of the honeydew was obtained after solution of the wax in CHCl_3 . Material collected from the Dural district of New South Wales yielded 80.6% honeydew, 7.0% insect bodies and 12.4% CHCl_3 -soluble material; that collected from the Gosford district of New South Wales yielded 85.8% honeydew, 1.7% insect bodies and 12.5% CHCl_3 -soluble material. While the material was

being collected it became apparent that the amount of honeydew produced by the insects varied from tree to tree. When clarified by treatment with charcoal in the cold, a sample of the honeydew exhibited insignificant optical activity.

The honeydew produced by *C. destructor* growing on native blackthorn (*Bursaria spinosa* Cav.) was obtained by the method described below.

From *Ceroplastes ceriferus* and *C. rubens*. The *C. ceriferus* occurred on *Dodonaea triquetra* Wendl. (native flax) growing in the Newcastle district (New South Wales) and the *C. rubens* on the leaves of citrus trees and of *Gordonia* trees growing in the Gosford district, the leaves of a lillipilly (*Eugenia luehmanni* F. Muell.) growing in the Sydney district and on the leaves of a mango (*Mangifera* sp.) growing in the Brisbane district (Queensland). Because of the small amounts of material available the method described above for *C. destructor* was modified as follows:

The waxy covering was carefully removed, leaving the insect attached to the twig or leaf, and dissolved in a small volume of CHCl_3 . The CHCl_3 solution was extracted with

Filter-paper partition chromatography

One-dimensional filter-paper partition chromatography was used for the detection of the sugars and sugar alcohols present in the honeydews. Whatman no. 1 filter-paper strips were used in both the 'descending' and the 'ascending' methods, and the experimental techniques employed were those described by Partridge (1948), Jermyn & Isherwood (1949) and Williams & Kirby (1948). As solvents the non-aqueous phases of the following systems were used (proportions are by vol.): *n*-butanol-ethanol-water (4:1:5); *n*-butanol-acetic acid-water (4:1:5) (Partridge, 1948); ethyl acetate-acetic acid-water (3:1:3); ethyl acetate-pyridine-water (5:2:5) (Jermyn & Isherwood, 1949). To detect the position of the individual sugars and sugar alcohols on the paper strips, use was made of the following four reagents: 1% (w/v) KMnO_4 in 2% (w/v) aqueous Na_2CO_3 (Pacsu, Mora & Kent, 1949), bromocresol purple in boric acid-borax solution (Bradfield & Flood, 1950), ammoniacal AgNO_3 (Partridge, 1948) and aniline hydrogen phthalate (Partridge, 1949).

Table 1. R_F values of sugar alcohols on Whatman no. 1 paper, corrected to 20°

Compound	Solvent systems (parts by vol.)		
	<i>n</i> -Butanol, 4 : ethanol, 1 : water, 5	<i>n</i> -Butanol, 4 : acetic acid, 1 : water, 5	Ethyl acetate, 3 : acetic acid, 1 : water, 3
	R_F	R_F	R_F
Inositol	0.07	0.06	0.08
Sorbitol	0.17	0.18	0.19
Dulcitol	0.17	0.16	0.18
Mannitol	0.20	0.19	0.19
Ribitol	0.28	0.25	0.28
Arabitol	0.26	0.22	—
Xylitol	0.24	0.20	—
Erythritol	0.31	0.35	0.34
Glucose*	0.185	0.18	0.17
Rhamnose*	0.345	0.37	0.34

* Published R_F values (Partridge, 1948; Jermyn & Isherwood, 1949).

10 ml. and 3 × 5 ml. water, the aqueous extract treated with charcoal, filtered and evaporated to dryness *in vacuo* at 40°. The residue was dissolved in 1 ml. water. By estimating the amount of CHCl_3 -soluble material and knowing the weight of insect bodies and the weight of the waxy covering it was possible to calculate that the *C. ceriferus* material contained 44.6% honeydew, 26.3% insect bodies and 29.1% CHCl_3 -soluble material; that of *C. rubens* growing on citrus contained 19.0% honeydew, 12.1% insect bodies and 68.9% CHCl_3 -soluble material. As with *C. destructor*, it is to be expected that these percentages will vary, depending upon a number of factors such as the time of the year when the insect material is collected and the tree upon which the insect is feeding. However, the values do reflect the nature of the scale insects and they show that *C. destructor* and *C. ceriferus* yield more honeydew and less wax than *C. rubens*. The last insect appears to have a much harder wax than the other two and since from published information (Hackman, 1951; Kono, 1932; Koyama, 1935; Chibnall, Piper, Pollard, Williams & Sahai, 1934) the composition of the waxes are not fundamentally different, the additional hardness appears to be due to the presence of less honeydew.

Since preliminary work had shown that the honeydews produced by the wax scales contained sugar alcohols, the behaviour of a number of representative alcohols under the chromatographic conditions used was studied. In Table 1 are listed the R_F values and the solvents used. The R_F values have been corrected to 20° (cf. Partridge, 1948) and adjusted to the published values of glucose and rhamnose, which two sugars were run on the same sheets of paper as the sugar alcohols. As indicated by Bradfield & Flood (1950) and by Hough, Jones & Wadman (1950) the R_F values for sorbitol, dulcitol and mannitol are about the same as that of glucose. The detecting agent used was the KMnO_4 - Na_2CO_3 reagent.

For each run 5 μ l. of honeydew extract were used. It was found convenient to run a series of paper strips of each extract in each solvent and to detect the sugars and/or sugar alcohols present on different strips by each of the reagents mentioned. In every case a mixture of glucose and ribitol was run as a control on the same strip of paper as were the honeydew extracts. Similar results were obtained with each of the four solvents used in the tests. Only one spot was obtained on the chromatograms with the honeydew from *C. destructor* and *C. ceriferus* and this was best detected with

the KMnO_4 or bromocresol purple reagents. Aniline hydrogen phthalate gave a negative result, thus showing the absence of aldopentoses, aldohexoses and uronic acids. Ammoniacal AgNO_3 did not give uniformly satisfactory results. The sugar alcohol present in the honeydew produced by these two insects proved indistinguishable from ribitol in all four solvents when run in parallel or when mixed together. Ribitol could be readily separated from mixtures with sugars. The honeydew from *C. rubens* gave three spots, two of which were detected with aniline hydrogen phthalate. The other spot corresponded to ribitol. One of the two reducing sugars corresponded to glucose, the other had R_F 0.08 at 20° in butanol-ethanol-water. The small amount of material available and the low concentration of this component prevented its further identification. For the three insects the results just mentioned were found to be independent of the host plant.

The approximate amount of ribitol present was determined by periodate oxidation (Hough, 1950; Jackson, 1944). The ribitol was extracted from the paper with water, oxidized with sodium metaperiodate and the resultant formic acid estimated using the procedure described by Hirst & Jones (1949). It was observed that the logarithm of the amount of ribitol was very approximately proportional to the area of the spot on the filter paper. Known quantities of ribitol were used to standardize the method. The honeydew from *C. ceriferus* was found to contain 5.8%, and that from *C. rubens* on lemon trees 3.0%, ribitol. The honeydews excreted by these three species also contained protein and amino-acids as detected by colour reactions.

When comparing the ribitol content of the honeydews excreted by the three *Ceroplastes* spp. a number of factors must be taken into consideration. The concentration of ribitol in the honeydew will vary with the amount of sooty mould present which utilizes the ribitol, and will depend upon the amount of water present. The *C. ceriferus* scale examined was apparently free from sooty mould, which may account for the high ribitol content. Both the *C. destructor* and *C. rubens* specimens were thickly covered with mould growth. The fact that *C. ceriferus* and *C. destructor* do not, as far as is known, occur on the same host plant introduces another variable, and the concentration of ribitol in the sap of the host plant may well be of major importance in determining its concentration in the honeydew.

Isolation and identification of ribitol

The clarified honeydew from the *C. destructor* material collected in the Dural district (190 g.) was evaporated to dryness at 40° *in vacuo* after the addition of CaCO_3 (0.5 g.) to preserve a neutral reaction. The residue was extracted with hot methanol (80 ml.); from the extract flat elongated prisms were deposited. A further crop of crystals was obtained from the mother liquor on standing. Yield 5.2 g. (2.7%). (The honeydew from the *C. destructor* material collected in the Gosford district yielded 3.1% ribitol.) After crystallization from water and drying at 80° , the material had m.p. 102° (uncorr.), not lowered by admixture of an authentic sample of ribitol. (Found: C, 39.3; H, 7.9. Calc. for $\text{C}_6\text{H}_{12}\text{O}_6$: C, 39.5; H, 7.9%.)

The dibenzylidene derivative was prepared by the method of Fischer (1893). After crystallization from ethanol and from acetone containing a little light petroleum the substance melted at 173° (uncorr.). The melting point recorded by Fischer ($164\text{--}165^\circ$) is lower, due probably to contamination

with the monobenzylidene derivative. (Found: C, 69.3; H, 6.1. Calc. for $\text{C}_{18}\text{H}_{20}\text{O}_6$: C, 69.5; H, 6.1%). Mixed melting point with an authentic sample of the dibenzylidene derivative of ribitol (m.p. 173°) was 173° (uncorr.).

Recognition of ribitol in plant sap of various species by paper chromatography

Twigs (without leaves; 25 g.) were cut into 1 in. lengths and extracted in a Waring Blendor with 25 ml. water. The material was filtered and the residue washed with 25 ml. water. The combined filtrate and wash water were mixed with decolorizing charcoal and filtered; CaCO_3 (0.5 g.) was added to the filtrate, which was evaporated to dryness at 40° . The dry residue was extracted with methanol and the methanolic solution evaporated to dryness. The residue was dissolved in water (2 ml.), mixed with charcoal and filtered. Portions of this extract (5 μl .) were used in the paper-chromatographic technique described above.

In this manner the presence of ribitol, glucose and other unidentified sugars was suggested in lemon, sweet orange and *Eugenia luehmanni* twigs. Glucose and other sugars, but no ribitol, could be detected in the twigs of *Eucalyptus globulus* (Labill.) var. *compactus*.

Ribitol in the honeydew of other scale insects

The honeydews produced by *Saissetia oleae* Bernard. (black or brown olive scale) growing on the twigs of a sweet orange tree, and by *Eriococcus eucalypti* Mask. growing on *Bursaria spinosa*, were extracted and examined by the methods described for the *Ceroplastes* scales. Ribitol, glucose and other sugars were indicated in the honeydews from both insects. However, by a similar method no ribitol could be detected in the honeydew excreted by *Eriococcus coriaceus* Mask. growing on the twigs of *Eucalyptus globulus* var. *compactus*.

DISCUSSION

Apart from the work described in this paper and the earlier reference by Fraser (1937), ribitol has been reported as occurring free in only two plants, *Adonis vernalis* L. (Podwykssozki, 1889; Merck, 1893), and *Bupleurum falcatum* L. (Wessely & Wang, 1938). However, in a combined form, it is a constituent of riboflavin. Evidence has now been obtained for the occurrence of ribitol in the honeydew produced by three of the four species of wax scale insects belonging to the genus *Ceroplastes* which occur in Australia: namely, *C. destructor* growing on citrus trees and on native blackthorn (*Bursaria spinosa*); *C. ceriferus* growing on native flax (*Dodonaea triquetra*); *C. rubens* growing on citrus trees, on a mango tree (*Mangifera* sp.), on a lillipilly (*Eugenia luehmanni*) and on a gordonia tree. Fraser (1937) has noted the occurrence of ribitol in the honeydew of *C. destructor* growing on *Melia azedarach* L. var. *australasica*. For *C. destructor* and *C. ceriferus* ribitol appears to be the only simple carbohydrate material present in the honeydew.

Filter-paper partition chromatography was used to detect the ribitol. Potassium permanganate in sodium carbonate solution, and bromocresol purple in boric acid-borax solution, were found to be more satisfactory reagents for detecting sugar alcohols than ammoniacal silver nitrate as used by Hough (1950). In the case of *C. destructor* proof for the identity of the sugar alcohol was obtained by isolating the ribitol and identifying it by physical methods and by preparation of the dibenzylidene derivative.

The sap produced by three of the above-mentioned host plants has been examined by paper chromatography for the presence of ribitol, and this or a similar sugar alcohol was detected in all cases. No attempt was made to identify any other constituent of the sap. Regarding other host plants, Fernald (1903), Froggatt (1921) and Zeck (1932) list a large number of plants as hosts for these *Ceroplastes* scales, representing a large number of families distributed amongst the ferns, gymnosperms and angiosperms (both monocotyledons and dicotyledons). The only similarity between these plants is that many would be classed as 'aromatic'.

Ribitol could not be detected, by the methods used, in the honeydews produced by all coccids; e.g. not from that of *Eriococcus coriaceus* Mask. growing on eucalypts (*Eucalyptus globulus* var. *compactus*) nor was it detected in the sap of the host plant. However, ribitol was found to be present in the sap of a sweet orange tree and in the honeydew produced by *Saissetia oleae* (black or brown olive scale) growing on it, and also in the honeydew produced by *Eriococcus eucalypti* growing on *Bursaria spinosa*.

It appears highly probable that scales of the genus *Ceroplastes* ingest and excrete the ribitol unchanged. It is not known if the insect metabolizes any of the ribitol and if so what kind of ribitol balance exists.

Drosophila melanogaster is able to utilize sugar alcohols including ribitol (Hassett, 1948). Fraser (1937) has shown that ribitol is a very satisfactory medium for the growth of the most important groups of sooty mould fungi, the Capnodiaceae and members of the Fungi Imperfecti. More satisfactory growth was made on a medium containing 2% of ribitol than on any other carbohydrate medium that was tested. It may well be that the species of sooty moulds found on plants affected by scales of the genus *Ceroplastes* depend upon their ability to utilize ribitol.

SUMMARY

1. The honeydews produced by three of the four species of wax scale insects belonging to the genus *Ceroplastes* which occur in Australia have been examined.

2. Ribitol was isolated from honeydew from *C. destructor* and identified.

3. By the use of filter-paper partition chromatography it has been indicated that the honeydews excreted by *C. destructor*, *C. ceriferus* and *C. rubens* contain ribitol. For *C. destructor* and *C. ceriferus* this appears to be the only simple type of carbohydrate material present. The honeydew excreted by *C. rubens* contains, in addition to ribitol, glucose and another (unidentified) reducing sugar.

4. The saps of three host plants for these scales were examined and found probably to contain ribitol.

5. The R_F values for a number of sugar alcohols in several solvents are given.

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