

228. *The Chemistry of Insects. Part II. Examination of the Woolly Aphis and of the White Pine Chermes.*

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THE woolly aphis, *Eriosoma lanigerum*, Hausmann, popularly known as American blight, is one of the most familiar and destructive of the British *aphididae*, though not originally a native of Britain. It excretes a protective tangle of long wax filaments (hence its popular name) and in most stages is deep purple. Some work on the chemistry of this insect has been done by Schulz (*Biochem. Z.*, 1922, **127**, 112) and Schulz and Becker (*ibid.*, 1933, **261**, 165; 1933, **264**, 87; 1934, **270**, 387). These authors are interested mainly in the wax, which they consider to be a mixed glyceride of palmitic acid (1 molecule) and myristic acid (2 molecules). They note the existence of a colouring matter, but do not attempt to isolate it, or determine its nature.

By the methods described later, little difficulty was experienced in isolating the crystalline colouring matter in a yield corresponding to 0.2% of the weight of the insects. The new substance, for which the name *lanigerin* is proposed, is orange in colour, gives orange-yellow, green-fluorescent solutions in ether and acetic acid, and dissolves in caustic alkali with a crimson colour. These reactions suggest that lanigerin is a polyhydroxyanthraquinone, and, if this is so, the green fluorescence must indicate a quinizarin grouping (Raistrick, Robinson, and Todd, *Biochem. J.*, 1934, **28**, 559). But the substance presents certain unusual features, particularly its very ready oxidation. The solid is stable; but in solution it is oxidised by contact with air, becoming at the same time darker in colour and soluble in alkali carbonate solution. The analytical figures are satisfied by the formula $C_{17}H_{14}O_5$, with three active hydrogen atoms; but naturally other formulæ are not definitely excluded. Attempts to prepare derivatives and degradation products have not yet been attended by any success, but they will be resumed when further supplies of the insects are available.

The opportunity also presented itself of examining the white pine chermes, *Adelges (Pineus) strobi*, Börner. This insect is less well known, but often causes considerable damage to plantations of the white pine. Like the woolly aphid, it secretes a protective wax; the insects are orange in colour.

No examination of this insect having previously been made, a considerable quantity was collected and worked up for both the wax and the colouring matter.

The wax (m. p. 106.1—106.4°), a detailed examination of which will be reported later, provides one of the relatively rare examples of a natural wax composed of a keto-alcohol esterified by a keto-acid. It is very similar to the cochineal wax examined by Chibnall, Latner, Williams, and Ayre (*Biochem. J.*, 1934, **28**, 313); but preliminary investigation has shown that it is not identical.

The colouring matter, isolated in a yield of under 0.08%, appears to belong to a type not hitherto found in *aphididae*. It has been named *strobimin*. Its analysis agrees best with the formula $C_{30}H_{24}O_8$, with three active hydrogen atoms. It is dark purple-brown and dissolves in organic solvents to give orange-crimson, yellow-fluorescent solutions, from which it is extracted by alkali with the formation of an emerald-green alkali derivative. A rather similar alkali colour reaction is given by certain hydroxyphenanthraquinones, e.g., 2-hydroxyphenanthraquinone, and theophoric acid (examined by Kögl, Erxleben, and Jänecke, *Annalen*, 1930, **482**, 105; it occurs in fungi of the *thelephora* species), which contains this grouping.

These investigations will be continued as supplies of the insects become available. Small amounts of other *aphididae* have also been examined, and in certain cases pigments of the hydroxyanthraquinone type appear to be present.

EXPERIMENTAL.

Examination of Eriosoma lanigerum, Hausmann.—The mixture of insects and wax was brushed off the twigs and stems of affected apple trees with a small soft paint brush. This material (30 g.) was treated with a few drops of chloroform to kill the insects and was then pounded with pumice powder and a few drops of acetic acid, the purple mass becoming khaki-brown. The colouring matter and wax were extracted by shaking with several changes of pure ether. The combined extract, fiery orange-red in colour and having a pronounced green fluorescence, was shaken with successive small quantities of ice-cold aqueous caustic potash (5%). At first only acetic acid was extracted; then the colouring matter was separated as a dark purple solution having an intense green fluorescence, and at the same time the ethereal solution became yellow. The latter was finally washed with water, dried, and evaporated, giving a residue of the crude wax which crystallised at once (2.4 g.).

The alkaline extracts were immediately acidified (the whole extraction and precipitation of the pigment should be carried out as rapidly as possible), and shaken with ether. The ethereal solution was dried over sodium sulphate and evaporated to a small bulk (about 3 c.c.), transferred to a centrifuge tube, and precipitated with light petroleum. An orange amorphous solid separated, and was collected by centrifuging and washed with light petroleum. The supernatant liquid on evaporation left a considerable amount of dark red, waxy material. When the orange solid was dissolved in a little ether and the solution slowly concentrated on the water-bath, *lanigerin* (60 mg.) crystallised. Purification of the substance gave considerable trouble until it was realised that its solutions are oxidised readily by contact with the air. The best method was to shake a solution of the substance in ether with sodium carbonate solution, which extracted the oxidation product but not the lanigerin. On drying and evaporation to a small bulk, as far as possible out of contact with air, lanigerin crystallised in small orange plates, m. p. 274—276° (decomp.) after darkening from 220° [Found, for material dried for 24 hours in a vacuum desiccator: C, 68.0; H, 4.8; OH (Zerewitinoff), 11.3. $C_{17}H_{14}O_5$ with three active hydrogen atoms requires C, 68.4; H, 4.7; OH, 11.4%]. It dissolves in ether and acetic acid to give orange-yellow solutions having a pronounced green fluorescence; addition of a drop of concentrated hydrochloric acid changes the colour to red. The sulphuric acid solution is scarlet and has no fluorescence. The crimson solution of the colouring matter in alkali fades on standing in the air. Attempts to acetylate lanigerin led to considerable darkening and the production of a dark purple, amorphous solid.

Examination of Adelges (Pineus) strobi, Börner.—The material was collected in Bagley Wood, near Oxford, in July, 1935. It consisted of the insects together with the excreted wax, and

contained in addition traces of bark débris. This material (120 g.) was heated to boiling with chloroform (about 800 c.c.), and the solution filtered rapidly (pump) through a warmed filter. This treatment was repeated, and the residue finally washed several times with hot chloroform. On cooling, the orange-brown filtrate deposited the wax (15 g., m. p. 105.5—106°), which was collected at 0° and well washed with cold chloroform. Twice crystallised from chloroform (charcoal), it formed small colourless plates, m. p. 106.1—106.4° (Found: C, 79.8; H, 13.0%).

The orange-brown filtrate from the wax was shaken with ice-cold aqueous caustic potash (5%), which extracted the pigment to give a purple-brown solution. The alkaline solution was separated and acidified, and the pigment taken up in ether.

The insect residues remaining after the chloroform extraction still contained a considerable amount of pigment. The material was mixed with pumice powder and a few drops of acetic acid, thoroughly ground, and shaken with ether. The extract was decanted, and the residue extracted several times until the extracts were colourless. From this ethereal solution the pigment was isolated by alkali extraction, and again taken up in ether.

The combined ethereal solutions were evaporated to a few c.c., and the pigment precipitated with light petroleum. It separated as a dark red, viscous liquid, which slowly solidified in a vacuum desiccator to a brittle resin (790 mg.). After some weeks hot alcohol (10 c.c.) was added. A clear solution was formed, which, however, soon began to deposit crystalline material. This was collected after some hours at 0° (92 mg., m. p. 226—230°). *Strobinin*, twice crystallised from chloroform-alcohol, formed small prisms generally grouped in spherical or dumb-bell shaped aggregates (62 mg.), m. p. 236—237° (decomp.). In the mass it had a dark purple, almost black, colour [Found, for material dried at 100° in a high vacuum: C, 70.4, 70.5; H, 4.5, 4.7; N, S, and O·CH₃ absent; OH (Zerewitinoff) 9.8, 9.4. C₃₀H₂₄O₈ with three active hydrogen atoms requires C, 70.3; H, 4.7; OH, 10.0%].

The substance is rather sparingly soluble in most organic solvents, particularly alcohol, giving orange-crimson solutions which exhibit a greenish-yellow fluorescence; the fluorescence is very marked in ultra-violet light, and then appears orange-yellow. In concentrated sulphuric acid strobinin forms an orange-scarlet solution with the same fluorescence. When its solution in acetic anhydride is treated with a trace of a solution of sulphuric acid in the same solvent, the colour changes during a few seconds from orange-crimson through brown and green to pure blue, and at the same time a remarkably intense red fluorescence develops. This solution is stable at the ordinary temperature, but on heating to the boiling point its colour immediately fades to pale brown. Strobinin is extracted from its ethereal solution by caustic alkali or alkali carbonate solution (but not by alkali bicarbonate) with the development of a bright emerald-green colour; after a few minutes an insoluble green precipitate separates, leaving a practically colourless solution. After several weeks the pigment could be regenerated from the alkaline suspension by acidification and shaking with ether.

The filtrate from the first crystallisation of the strobinin was examined for other pigments, but no crystalline material could be obtained, either directly or after acetylation.

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