Colouring Matters of the Aphididæ. Part XI. Pigments from* Hamamelistes *Species.*

By S. F. MacDonald.

[Reprint Order No. 4118.]

The pigments of *Hamamelistes spinosus*, Shimer, and *H. betulae*, Mordvilko, represent a new type of aphin series. The native pigments (heteroaphins) of these aphids are converted enzymically into the same rhodoaphin, $C_{30}H_{22}O_{11}$.

The rhodoaphin has been reduced to a typical erythroaphin, and comparison of the rhodoaphin with hydroxy- and dihydroxy-erythroaphin-*fb* suggests that it is a dihydroxyerythroaphin containing a further oxygen atom of unknown function.

Aphins so far described (Duewell, Human, Johnson, MacDonald, and Todd, *J.*, 1950, 3304) have belonged to aphin series of the type first described by Sorby [*Quart. J. Microscop. Sci.*, 1871, II (N.S.), 352]. The native pigments, protoaphins, were converted enzymically into xanthoaphins and thence by chemical methods successively into chrysoaphins and erythroaphins. Though minute amounts of the four pigments were readily detected and distinguished, the corresponding pigments (*e.g.*, erythroaphins) from different species could be clearly distinguished only by *X*-ray powder photographs and infra-red spectra.

The only other aphid pigments found which were apparently related to these aphins were those of *Hamamelistes* species, which resembled the erythroaphins in colour and spectra but lacked the usual precursors. Work was begun with the North American *Hamamelistes spinosus*, Shimer, and continued first with the British *H. betulae*, Mordvilko, then with *H. spinosus* again. Both are primitive aphids found, surrounded by masses of wax and sugar, in shallow galls opening on the under side of leaves of the white birch. They are unusual among aphids in that their wings are horizontal when at rest. The methods of Duewell *et al.* (*loc. cit.*) for the preliminary examination had to be modified to give adequate separation of the pigments by partition. The only native pigment detected was the heteroaphin, a water-soluble red pigment occurring in the hæmolymph in amounts representing about 1% of the live-insect weight. It is unstable in extracts unless enzymes have been inactivated by heat or organic solvents. In unstabilized extracts, and in stabilized extracts to which the freshly pulped insects have been added, heteroaphin is rapidly converted into rhodoaphin, a second red pigment which is soluble in ether. These names imply a relation to the previously known aphin series which is justified by the work described below. In accordance with the convention (Duewell, Human, Johnson, MacDonald, and Todd, *Nature*, 1948, **162**, 759), suffixes are added to indicate the insect source: *sp* from *H. spinosus* and *be* from *H. betulae*.

Except in their solubility, the heteroaphins-*sp* and -*be* resemble erythroaphin rather than any of its precursors, becoming green reversibly with alkali and showing visible absorption (Table 1). Although their enzymic conversion into rhodoaphins parallels that of protoaphin-*ph* into xanthoaphin-*ph*, *H. betulae* pulp does not bring about the latter change. Also, these heteroaphins are converted into rhodoaphins by mild acid, but the conversion of protoaphins into xanthoaphins can only be carried out enzymically and appears to be complex (*J.*, 1952, 4925). Although there are no apparent differences between the heteroaphins-*sp* and -*be*, it is uncertain whether or not they are identical for they have not been obtained crystalline.

Solutions of the rhodoaphins, obtained when *H. spinosus* or *H. betulae* were crushed a few minutes before extraction, were strikingly similar to those of the erythroaphins in colour and spectra (Table 1); they also became green in alkali but differed in being unstable in concentrated sulphuric acid. However, methods applicable to the erythroaphins

* Part X, *J.*, 1954, 1285.

resulted in a poor yield of crystalline rhodoaphin-*sp* and failed to give crystalline rhodoaphin-*be*.

Unsuccessful attempts were made to show that the rhodoaphins were oxoerythroaphins by making and comparing the oximes. In these experiments with erythroaphin-*fb*, spectra resembling that of the rhodoaphins were noted when hot solutions of the erythroaphin were treated with ammonia and air, and the product was isolated as diaminoerythroaphin-*fb* (Part VII, Brown, Johnson, MacDonald, Quayle, and Todd, *J.*, 1952, 4928). Though this product did not resemble the rhodoaphins in basicity and stability, it was readily converted into dihydroxyerythroaphin-*fb* which did. A hydroxyerythroaphin-*fb* was also obtained for comparison, though no trihydroxyerythroaphin could be prepared (Part VII, *loc. cit.*). Comparison of the spectra of these pigments (Table 1) suggested that the rhodoaphins might be trihydroxyerythroaphins. In alkali, 60% perchloric acid, under reducing conditions (acid or alkaline), and after conversion of the pigments into metal complexes (particularly those with nickel), the spectra showed the same regularity but none were so characteristic as those in chloroform.

TABLE 1. *Absorption maxima* (mμ).

Erythroaphin-*fb*	(in $CHCl_3$) 589, 563, 521, 485, 447, 421
Hydroxyerythroaphin-*fb*	(in $CHCl_3$) 592, 563, 523, 488, 450, 425
Dihydroxyerythroaphin-*fb*	(in $CHCl_3$) 595, 567, 526, 490, 453, 426
Rhodoaphin-*sp* and -*be*	(in $CHCl_3$) 596, 568, 528, 454, 427—429
Heteroaphin-*sp* and -*be*	(in MeOH) 551, 515, 443

The rhodoaphins were then found to decompose, like dihydroxyerythroaphin-*fb* (Part VII, *loc. cit.*), when their solutions in chloroform or ether were shaken with water, with buffers of pH 5—8, or with aqueous sodium hydrogen carbonate, the red-brown product separating at the interface. Stronger alkali extracts the rhodoaphins as their green salts, and the pigments are returned unchanged to the organic layer after acidification. Satisfactory yields of the crystalline rhodoaphins-*be* and -*sp* were then obtained by maintaining acid conditions during their extraction. Their identity was established by *X*-ray powder photographs and infra-red spectra. Analysis of rhodoaphin-*be* indicated a formula $C_{30}H_{22}O_{11}$ which is that of a trihydroxyerythroaphin. These rhodoaphins show hydroxyl absorption in their infra-red spectra as do hydroxy- and dihydroxy-erythroaphin-*fb*; the erythroaphins do not.

The pigments also show gradations in stability and partition behaviour (Table 2), in acidity, and in the speed with which they give Blount's colour reaction with acetic anhydride and mineral acid. Unlike the spectra, none of these methods will distinguish the rhodoaphins from dihydroxyerythroaphin-*fb*. As their partition behaviour is not that expected in a trihydroxyerythroaphin, the rhodoaphins are regarded as a dihydroxyerythroaphin having a further oxygen atom with some other function.

TABLE 2.

	Stability			Partition		
	In conc. H_2SO_4	In pyridine at 100°	In $CHCl_3$ with aq. $NaHCO_3$	% H_2SO_4 extracting pigment from $CHCl_3$	% Aq. resorcinol extracting pigment from $CHCl_3$	Partition between CS_2 and MeOH : % in MeOH
Erythroaphin-*fb*	Stable	Rapid decomp.	Stable	72	>66	<3
Hydroxyerythroaphin-*fb*	Slow decomp.	Slow decomp.	Stable	*ca.* 68	*ca.* 50	*ca.* 20
Dihydroxyerythroaphin-*fb* and Rhodoaphin-*sp* and -*be*	Rapid decomp.	Stable	Decomp.	<72	33	*ca.* 85

The removal of hydroxyl groups from anthraquinones (Dimroth and Fick, *Annalen*, 1916, **411**, 315) suggested reduction of the rhodoaphins to an erythroaphin. After model experiments with hydroxy-, diamino- and dihydroxy-erythroaphin-*fb* (Part VII, *loc. cit.*), the rhodoaphins were reduced to typical erythroaphins as judged by their spectra,

stability, amination, and partition behaviour. These were not obtained in an obviously crystalline condition, and the 6—10 μ regions in their spectra suggested that they might be impure erythroaphin-*fb*.

Experimental

Spectra were measured in chloroform which had been washed several times with concentrated sulphuric acid, kept for some hours over potassium hydroxide, distilled, and stabilized by the addition of pure dry ether (5% v/v). When pure but unstabilized chloroform was used, the optical densities in the ultra-violet region increased rapidly during measurement, apparently because of the formation of hydrogen chloride. When Beckman or Unicam instruments were used, the two small peaks at *ca.* 490 and *ca.* 595 mμ were not always detected in solutions of dihydroxyerythroaphin-*fb* and the rhodoaphins. These at *ca.* 595 mμ were measured visually when necessary; their sharpness makes them the most convenient guide in work with these pigments.

Preliminary Examination of the Insects.—The water and methanol used contained tartaric acid (0·5%). About 15 live insects were ground with methanol (2 drops); when water (1·5 c.c.) and ether (1·5 c.c.) were added to the mixture the red heteroaphin remained in the aqueous phase, and the nearly colourless ether layer contained only traces of pigment. The live insects were ground with water (1 drop) and, after being kept for 1 min. for fermentation, stirred with methanol (2 drops); after addition of ether and water as before, the aqueous phase was nearly colourless and contained only traces of pigment, the red rhodoaphin being in the ether.

Non-enzymic Conversion of Heteroaphin.—Live *H. betulae* were ground with methanol, and 10% hydrochloric acid (1·5 c.c.) was added. After a few minutes, ether and water were added; only traces of pigment were found in the aqueous layer, but rhodoaphin-*be* was identified in the ether by its colour and spectrum.

Rhodoaphin-sp *from* H. spinosus.—(i) The insects (5 g.; preserved for 6 weeks at 0°) were freed from wax and sugar by, successively, acetone, ether, benzene, and acetone. The pigment was extracted by grinding and filtering with, successively, acetone, water, and 80% acetone. The last extract, containing the bulk of the pigment, was evaporated *in vacuo* and the residue extracted with ether. After being washed with water, the ethereal layer was evaporated and the residue crystallized from hot ethanol. Rhodoaphin-*sp* formed small rods (*ca.* 2 mg.).

(ii) By the method given for rhodoaphin-*be*, the insects (6 g.) gave rhodoaphin-*sp* (25 mg. crude, 4·8 mg. crystalline) (Found: N, 0·0%). The infra-red spectrum of a mull in Nujol showed maxima at 632, 720, 838, 854, 881, 943, 975, 1048, 1076, 1115, 1155, 1175, 1203, 1230, 1255, 1292, 1304, 1571, 1633, 2720, 3270 cm.$^{-1}$; in the hydroxyl region there was also a shoulder at 3170 cm.$^{-1}$.

Rhodoaphin-be *from* H. betulae.—The live insects (3·8 g.) were repeatedly washed with light petroleum (b. p. 40—60°) by decantation to remove excess of wax, ground with aqueous tartaric acid (0·5%), allowed to ferment for a few minutes, and extracted with aqueous methanol (85%, acidified with tartaric acid; 3 × 30 c.c.). The methanol was washed with light petroleum (b. p. 40—60°), water was added, and the pigment extracted from the aqueous methanol with ether (total, 60 c.c.). The ether was diluted with light petroleum (b. p. 40—60°; 2 vols.), filtered, and concentrated under slightly reduced pressure. The granular crude product (20 mg.) which separated was extracted with ether (10 c.c.; thimble), ethanol (1 c.c.) was added, and the extract concentrated and kept for 5 days at 0°. *Rhodoaphin*-be separated as fine red needles (7·2 mg.) and the mother liquors gave more (1·6 mg.) of the well-crystallised material (Found: C, 64·5; H, 4·3. $C_{30}H_{22}O_{11}$ requires C, 64·5; H, 4·0%).

Erythroaphin.—(i) *From rhodoaphin*-be. Amorphous rhodoaphin-*be* from the mother-liquors from the crystalline product above, and crude granular rhodoaphin-*be* (7·8 mg.) prepared as above from *H. betulae* (1·1 g.) were available. Both specimens had maxima at 596 μ and were destroyed by the treatment with bicarbonate, so related pigments were absent.

The combined specimens were reduced and the product purified exactly as in the analogous reduction of dihydroxyerythroaphin-*fb* (*J.*, 1952, 4932). The erythroaphin (3 mg.) separated from chloroform–ethanol in granular form. Absorption maxima in $CHCl_3$: 588—589, 563, 521, 447, 423—424, 335, 318, 255 mμ; min. at 581, 536, 499, 431, 361, 328, 314 mμ.

(ii) *From rhodoaphin*-sp. Amorphous rhodoaphin-*sp* (20 mg.) from the mother-liquors from the crystalline product was reduced and the product purified as in (i) except that the chloroform solution of the erythroaphin was also washed with 50% aqueous resorcinol. The product was granular. Absorption max. in $CHCl_3$: 588, 563, 521, 486, 448, 422 mμ.

Its identity was confirmed by 1 hour's heating at 90° in aqueous dioxan containing nitrobenzene and ammonium carbonate, giving a diaminoerythroaphin (*J.*, 1952, 4928), identified in the comparison spectroscope and by extraction from chloroform into 10% hydrochloric acid.

The author is grateful to Dr. W. Cochran for the *X*-ray powder photographs, to Dr. R. N. Haszeldine and Dr. R. N. Jones for visible and infra-red spectra, and to Mr. H. L. G. Stroyan who identified the insects. Grateful acknowledgment is made to the Wellcome Trustees for a Fellowship, during the tenure of which part of this work was carried out.

UNIVERSITY CHEMICAL LABORATORY, CAMBRIDGE.
DEPARTMENT OF MEDICAL RESEARCH, UNIVERSITY OF TORONTO. [*Received February 21st*, 1953.]