644. *Colouring Matters of the Aphididæ. Part IV. A General Survey of the Occurrence of the Aphins.*

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The distribution of aphins and other colouring matters in many species of Aphididæ is surveyed. A rapid method for the detection of aphins in the presence or absence of non-aphin colouring matters is outlined. Further procedures have been developed for the detection and separation of the individual aphins from complex pigment mixtures. The isolation of aphins from *Aphis sambuci* L., *Eriosoma lanigerum*, *Myzus cerasi* F., *Sappaphis pyri* (Fonsc.), and *A. philadelphi* is described.

The isolation of closely related series of aphid pigments or aphins from two species of Aphididæ, *Aphis fabæ* and *Tuberolachnus salignus*, has been reported in Parts I—II (Duewell, Human, Johnson, MacDonald, and Todd, *Nature*, 1948, **162**, 759; *J.*, 1950, 477, 485). The extent to

which compounds of this nature occur in a number of other aphid species is now recorded. The table lists some twenty species in which aphins have been detected and includes several from which typical aphid pigments have been isolated in crystalline form. A list of aphid species in which aphins have not so far been detected is also given. Nearly all the aphid species which have been examined contain non-aphin pigments in addition and, in the species recorded, the large majority of the observed pigments in the living insects belong to one or more of three classes: protoaphin (in 45% of the species), yellow or brown non-aphin pigments (in 50%), and green non-aphin pigments (in 60%). The amount of protoaphin present is normally about 0·5% of the live weight of the insect but varies between 2·5 and 0·02%, the latter figure representing the limit of sensitivity of the test used; it is possible that even smaller quantities of protoaphin may be present in some of the species listed by us as giving negative tests.

A system has been elaborated for the examination of aphids whereby protoaphin can be detected in the presence of non-aphin pigments, and at the same time xanthoaphin, chrysoaphin, and erythroaphin can be shown not to occur in living insects but to arise successively from the protoaphins by the series of changes described in the previous papers. To observe these transformations of the protoaphins, it is necessary to use samples containing only living insects and for this reason methods have been so chosen that only small quantities of aphids need be used in the test, which involves a comparison of the colour, absorption spectra, and partition behaviour of the pigments from fermented and unfermented insects. A qualitative examination by this method of each aphid species should be carried out before any isolation experiments as certain of the non-aphin pigments may interfere with the isolation of aphins by the standard procedures (Part II, *loc. cit.*). In the early stages of our investigations of the Aphididæ, after the isolation of the easily recognised fluorescent aphins, it was this method of examination which established protoaphin as their ill-defined, water-soluble precursor; nothing was known at that time of its stability and even its colour was usually masked by other pigments. It was then found that, after removal of acetone from unfermented extracts of *A. philadelphi*, the protoaphin-*ph* therein could be converted into xanthoaphin by adding the fresh insect pulp; the xanthoaphin was then readily recognised spectroscopically after it had been extracted from the mixture with ether. Solubility and fermentation by added insect pulp in neutralised aqueous solution thus provided a method for identifying protoaphin and for demonstrating the presence of intact protoaphin in the unfermented acetone extract; the method also permitted its stability to be investigated and the course of its purification and isolation to be followed. In surprising contrast to xanthoaphin, protoaphin proved relatively stable to heat, acid, or alkali; insects difficult to separate from the host could therefore be heat-killed to destroy enzymes, thus avoiding the use of large volumes of solvent in the preparation of protoaphin extracts. Finally, it was found that the non-aphin pigments which accompany protoaphin-*ph* in extracts of *A. philadelphi* could be separated by preferential adsorption on Darco G.60 charcoal, and the fortunate choice of this acid charcoal lowered the pH to the point where protoaphin-*ph* could be crystallised. The same method was applied without modification to *A. fabæ* (Part II, *loc. cit.*) although with *T. salignus* (Part III, *loc. cit.*) even a considerable modification gave poor results.

We have previously remarked on the similarities between the corresponding aphins from different species, and the close relationship between the protoaphins has been further demonstrated by a number of " cross-fermentations " of the protoaphin (either the crystalline compound or heat-stabilised insect extracts) from one species with the enzymes from others. Thus the crystalline protoaphin-*ph* in buffered phosphate solution at pH 6·5 was converted into xanthoaphin-*ph* by small amounts of freshly pulped *A. sambuci* or *T. salignus*. No conversion was brought about by species containing no protoaphin, *e.g. Hamamelistes betulæ*, from which it appears that the enzyme concerned occurs only in those insects which contain protoaphin. This fact has been used as an aid in the detection of very small quantities of protoaphin in insect samples, the fermentation being carried out in the presence of added protoaphin which increases the sensitivity of the test some twenty- to thirty-fold (*i.e.* making it possible to detect amounts of protoaphin $\geqslant 0{\cdot}001\%$); it has been shown previously (Part II, *loc. cit.*) that one insect contains sufficient enzyme to convert many times its own content of protoaphin. The converse also holds—protoaphin appears to be accompanied always by the enzyme in the living insect, for aphids containing pigments resembling protoaphin but giving no xanthoaphin on fermentation still fail to do so when fermented by the pulp of a species known to contain the enzyme.

A number of methods have been developed to supplement the standard ones when complex mixtures of pigments are encountered. Thus considerable use has been made of the partitioning

of erythroaphin between chloroform and sulphuric acid (Part III, *loc. cit.*) for the purification of this pigment, and in this connection the absorption spectrum of erythroaphin in concentrated sulphuric acid solution provides a most sensitive test for the fluorescent aphins, as the band at 5680 A. can be detected at concentrations as low as 4×10^{-7}. Although xanthoaphin and chrysoaphin are not sufficiently stable to be fractionated between chloroform and sulphuric acid, they, as well as erythroaphin, can be freed from fats, waxes, and non-aphin yellow pigments by partition between aqueous solutions of resorcinol and solvents in which the latter is not appreciably soluble, *e.g.* light petroleum, chloroform, or benzene. Xanthoaphin is completely extracted by 50% resorcinol from chloroform, chrysoaphin by 66%, but erythroaphin is only extracted completely by 66% resorcinol after the chloroform has been diluted with an equal volume of light petroleum. The aphins can be returned to fresh solvent quantitatively after dilution of the resorcinol extract with water. On a small scale, the method can be used to separate the three pigments by fractionation. It is most useful with fatty petroleum extracts, where great volume concentration can be attained without emulsification; here, the light petroleum is first freed from acetone, if present, by extraction with saturated neutral aqueous sodium iodide. The solvent power of aqueous phenol, catechol, resorcinol, and pyrogallol has been utilised previously (Friedländer, D.R.-P. 199,690; *Chem. Zentr.*, 1908, II, 359; Hochstetter, D.R.-P. 268,452; *Chem. Zentr.*, 1914, I, 310); the efficiency and utility of phenol is diminished by its greater tendency to partition between the aqueous and the organic phase. We have observed that aqueous resorcinol can also be used in a similar way in the extraction of porphyrins and hydroxyanthraquinones.

All aphin and non-aphin pigments present in aphids are extracted by *n*-butanol and adsorbed by charcoal (Darco G.60) from slightly acid aqueous solution. Only protoaphin can then be efficiently eluted from the charcoal, and all pigments except protoaphin are adsorbed on Darco G.60 from 60% aqueous acetone or aqueous methanol. Of the fluorescent aphins, erythroaphin is most readily adsorbed and xanthoaphin least; the latter can be freed from erythroaphin and chrysoaphin by shaking and centrifuging 75% acetone or methanol extracts with successive small amounts of Darco G.60. Of the three fluorescent aphins, xanthoaphin is the most acidic, erythroaphin the least. Xanthoaphin can be separated on a small scale by extracting it from ether with very dilute ammonia; it must be returned at once to fresh ether, citric acid being used to neutralise the ammonia, otherwise the yellowish alkaline solution acquires a crimson tint owing to the formation of chrysoaphin.

Our observations on the various aphin-containing species which have been identified with certainty are listed in the table which extends and corrects the previous list of isolated pigments (Part I, *loc. cit.*). Aphins have also been observed in some twenty additional species which we refrain from listing because of the uncertainty in their identification.

Species (host).	Proto-aphin.	Xantho-aphin.	Chryso-aphin.	Erythro-aphin.
Adelges (*Pineus*) *strobi* (Börner) (White pine) [1,3]	—	—	—	i
Adelgid (unidentified) (White pine) [2]	+	+	+	+
Aphis fabæ Scop. (beans) [1,2,4]	i	i [6]	i [7]	i [8]
A. rumicis L. (docks) [1]	+	+ [6]	+	+
A. philadelphi (Börner) (philadelphus and burdock) [2]	i	+ [6]	+ [7]	+ [8]
A. viburni Scop. (snowball) [1,2]	+	+ [6]	+ [7]	+ [8]
A. cognatella (M. G. Jones) (spindle) [1]	+	+ [6]	+ [7]	+ [8]
A. hederæ Kalt. (ivy) [1]	+	+ [6]	+ [7]	+ [8]
A. sambuci L. (elder) [1]	+	i [6]	i [7]	i [8]
A. ilicis Kalt. (holly) [1]	—	+	+	+
Brachycaudus klugkisti (Börner) (campion) [1]	+	+ [6]	+ [7]	+ [8]
B. rociadæ (Cockerell) (delphinium) [2]	+	—	—	+
Dactynotus (*Uromelan*) *jaceæ* (L.) (knapweed) [1]	+	+	+	+
Eriosoma lanigerum (Hausmann) (apple tree) [1,2,3]	+	+ [6]	i [7]	i [8]
E. ulmi (L.) (elms) [1]	+	+ [6]	+ [7]	+ [8]
Myzus cerasi (F.) (cherry) [1,2]	+	+ [6]	i [7]	i [8]
Rhopalosiphum nymphaeæ (L.) (arrow head) [2]	—	+	+ [7]	+ [8]
Sappaphis pyri (Fonsc.) (pear) [1]	—	i	+	i
Schizolachnus tomentosis (de Geer) (Scots pine) [1]	+	+ [6]	—	+
Tuberolachnus salignus (Gmelin) (willow) [1,5]	i	i [6]	i [7]	i [8]

i = isolated; + = detected; — = not looked for or observation prevented.

[1] Collected in Britain. [2] Collected in North America. [3] Blount, *loc. cit.* [4] Part II, *loc. cit.* [5] Part III, *loc. cit.* [6] Xanthoaphin observed only after fermentation and then free from chrysoaphin and erythroaphin. [7] Chrysoaphin observed only after treatment of xanthoaphin with formic acid at room temperature and then free from erythroaphin. [8] Erythroaphin observed only after treatment of chrysoaphin with hydrochloric acid.

There can be no doubt that Blount's lanigerin from *Eriosoma lanigerum* (*J.*, 1936, 1034) is an artefact derived from a protoaphin, and his description of it suggests a chrysoaphin which, when later examined by Dhéré (*Compt. rend. Soc. Biol.*, 1939, **131**, 672), had become a mixture of a chrysoaphin and an erythroaphin. We have confirmed the isolation of chrysoaphin-*ln* (lanigerin) and in addition have prepared erythroaphin-*ln* from this species, which contains only traces of non-aphin pigments. Other new aphins to be isolated include xanthoaphin-*sm*, chrysoaphin-*sm*, and erythroaphin-*sm* from *A. sambuci* L.; xanthoaphin-*ce*, chrysoaphin-*ce*, and erythroaphin-*ce* from *Myzus cerasi* (F.); and xanthoaphin-*py* and erythroaphin-*py* from *Sappaphis pyri* (Fonsc.).

The aphins of the *Adelginæ* are of particular interest because these insects belong to the family Phylloxeridæ; otherwise aphins have so far been found only in Aphididæ. Strobinin, which Blount (*loc. cit.*) isolated from *Adelges* (*Pineus*) *strobi* (Börner) parasitising white pine, is an erythroaphin as shown by the spectroscopic examination of a sample of his product. There is no reason to doubt that it stands at the end of a typical aphin series, but as yet samples of this insect species have not been available to us, although we have detected all four aphins in an unidentified adelgid found on white pine. There are special difficulties in working with adelgids for we have not found them in dense colonies; they are difficult to identify, and it is not easy to collect them in an undamaged state and to distinguish living from dead insects.

In the survey given in the table, the inter-relationship between the protoaphins, xanthoaphins, chrysoaphins, and erythroaphins in each species has been established whenever the state of the sample has permitted; where these relationships have not been rigorously established, the pigments have been listed as detected without qualification. Aphin pigments were not detected in the following species: *Aphis urticaria* (Kalt.) (nettles), *Brevicoryne brassicæ* (L.) (cabbage), *Chaitophorus populicola* (Thomas) (poplar), *Cryptomyzus ribis* (L.) (currants), *Dactynotus cirsii* (L.) (thistles), *Drepanosiphum plantanoides* (Schrank) (sycamore), *Hyadaphis fœniculi* (Pass.) (honeysuckle), *Hyalopterus arundinis* (F.) (Victoria plum), *Liosomaphis berberidis* (Kalt.) (barberry), *Macrosiphum gei* (Koch) (umbelliferæ), *M. albifrons* Essig (lupins), *M. rosæ* (L.) (cultivated roses), *Microlophium schranki* (Theobald) (nettles), *Myzaphis rosarum* (Kalt.) (roses), *Nasonovia ribis-nigri* (Mosley) (currants), *Pemphigus bursarius* (L.) (poplar), *Periphyllus aceris* (L.) (sycamore), *Prociphilus tessellatus* (alder), *Sappaphis viburnicola* (Gillette) (viburnum), and *Symydobius oblongus* (v. Heyd) (birch).

The primitive species, *Hamamelistes spinosus* (Shimer) and *H. betulæ* (Mordrelko) contain pigments which bear some relation to the aphins and these will form the subject of a separate communication. Of the non-aphin pigments which have been encountered in the course of this work, mention has already been made of the petroleum-soluble yellow or brown pigments and the water-soluble green pigments. The former often showed an absorption spectrum with one to three lines in the blue and green regions of the spectrum; they were usually non-fluorescent and differed from the aphins in being extractable from acetone by light petroleum before as well as after fermentation. It is possible that some of these may be carotenoids; Sorby [*Quart. J. Microscop. Sci.*, 1871, ii (N.S.), 352] compared pigments of this type to the colouring matters of fats and oils. Some of the water-soluble greenish pigments are strongly fluorescent but others are non-fluorescent. Solutions are changed from green to yellow reversibly in the commonest type, *e.g.* from *A. fabæ* or *A. sambuci*, and in some cases mild acid hydrolysis gives ether-soluble green pigments. Like the previous group, they are usually unaffected during the fermentation of protoaphin.

We have not confirmed our preliminary observations on *Dactynotus jaceæ* and *D. cirsii* (Part I, *loc. cit.*). The protoaphin content of *D. jaceæ* is very small and aphins could not be detected in *D. cirsii* with the present test. The pigment obtained from these species is essentially a mixture of one of the above-mentioned yellow non-aphin pigments, which spectroscopically simulates chrysoaphin to some extent, and a red pigment which has no characteristic spectrum and is easily washed out of a light petroleum solution with 25% aqueous methanol or concentrated hydrochloric acid.

Experimental.

Unless otherwise stated, light petroleum refers to that fraction of b. p. 40—60°.

Preparation of Samples for Preliminary Examination.—Wherever possible, samples were taken from dense and apparently homogeneous colonies; where these proved to consist of mixed species, the results of any examination were ignored. Samples consisted of live insects as far as possible; where it was necessary to preserve the insects before identification, a representative selection, preferably containing a variety of forms, was taken and suspended in a mixture of 90% ethanol (2 vols.) and 75% (w/w) lactic

acid (1 vol.). It was also of value to keep samples of the host plants as the differentiation of closely related species was aided by the knowledge of the hosts as well as the date of collection.

The Examination of Insects for Pigments.—Living insects (about 5 mg.; 1—20 individuals) were crushed and stirred in 80% acetone (0·5 c.c.) in a small test-tube, light petroleum (0·5 c.c.) was added, the mixture shaken and centrifuged, and the two layers (*A* and *B* = aqueous and light petroleum layers, respectively) separated. A second similar sample of insects was crushed in phosphate buffer, pH 6·5 (0·3 c.c.) and the mixture kept for 3 minutes. Acetone (0·5 c.c.) and light petroleum (0·5 c.c.) were added and the layers *C* and *D* (aqueous and light petroleum layers, respectively) separated as before. The various extracts were then examined, colour, fluorescence, and spectra (hand spectroscope) being noted, after dilution if necessary.

In the rare cases when protoaphin was the sole pigment of the insect, layer *A*, which contained protoaphin, was red with no fluorescence and was reversibly changed to yellow on the addition of acid. Layers *B* and *C* contained no pigment and layer *D*, which contained xanthoaphin, was yellow with a greenish-yellow fluorescence and showed absorption bands at 4330 and 4620 A. The presence of xanthoaphin was confirmed by its conversion into chrysoaphin (yellow fluorescence; bands at 4570 and 4880 A.) on the addition of a little cold formic acid and into erythroaphin (orange fluorescence; bands at 5890, 5640, 5210, and 4520 A.) with concentrated hydrochloric acid, preferably after a preliminary washing of the petroleum layer with water to remove acetone and formic acid. The erythroaphin frequently crystallised at this stage leaving the solvent almost colourless and it could also be separated as its insoluble green sodium salt by shaking with aqueous sodium hydroxide. Very small quantities of aphins were best detected by observing the spectrum in sulphuric acid (cf. erythroaphin-*sl*, Part III, *loc. cit.*) and confirmed by partition between 64—72% sulphuric acid and chloroform (Part III, *loc. cit.*). If the time of fermentation was too short for complete conversion, some protoaphin was observed in layer *C*, and if too long, chrysoaphin as well as xanthoaphin was found in *D*. When dead insects were present in the sample, both light petroleum layers *B* and *D* often contained chrysoaphin and erythroaphin as well as xanthoaphin, and the aqueous layer *C* contained certain ill-defined non-fermentable degradation products of protoaphin.

In most cases, however, other non-aphin pigments were also present. The green water-soluble pigments, being non-fermentable, appeared in both aqueous layers, mixed with protoaphin in *A* and free from protoaphin in *C*. They could also be separated from protoaphin by means of their preferential absorption on charcoal (Darco G.60) from aqueous acetone.

The yellow or brown light petroleum-soluble non-aphin pigments usually appeared in both layers *B* and *D*. They were unaffected by the action of alkali or acids, thus permitting the aphin changes to be followed by observations of the relative intensities of the absorption bands. When only small quantities of the aphins were present it was preferable to separate them by extraction with aqueous resorcinol, and then to re-extract the aphins from the diluted resorcinol solution with the organic solvent.

Isolation of Aphins.—Protoaphin-ph. Infested tops of burdock collected the same day were placed in water at 70° for 3 minutes. The insects were then separated on muslin, put through a coarse sieve to remove parasites and drained on a Buchner funnel (no paper) moderate suction being used. The insects (380 g.; 80% moisture content; equivalent to 310 g. of live insects with 75% moisture content; about 8×10^5 individuals) were diluted with methanol (to 1000 c.c.) and ground in a Waring Blendor for 5 minutes. The product was separated in a centrifuge and the pulp diluted with 60% acetone (to 1000 c.c.) and again ground and centrifuged. Hyflo Supercel was stirred into the extracts which were then filtered, the filtrate diluted with an equal volume of methanol and extracted with light petroleum (2×200 c.c.). The acetone–methanol layer was concentrated under reduced pressure (to 700 c.c.), cooled, and stirred with active charcoal (Darco G.60; 15 g.), which was then separated off. The filtrate was concentrated (to 200 c.c.), stirred with more charcoal (10 g.) which was again separated off, and the combined charcoal adsorbates well washed with water and eluted with 75% acetone (700 c.c.). The eluate was concentrated (to 200 c.c.) under reduced pressure, whereupon *protoaphin*-ph separated as tan-coloured crystals (1·66 g.) which were removed in a centrifuge, washed with water, and dried *in vacuo*. Concentration of the mother-liquors, followed by re-adsorption on charcoal (5 g.) and elution as before, gave a further quantity (0·19 g.) of a less pure product. For analysis, the protoaphin was recrystallised from aqueous methanol, dissolved in aqueous sodium hydroxide (N/100), the solution filtered, the filtrate acidified with hydrochloric acid (N/100), and the precipitate finally recrystallised again from aqueous methanol (Found, in material dried at 56°/0·1 mm.: C, 55·7; 55·6; H, 6·1, 6·1. $C_{36}H_{44}O_{19}$ requires C, 55·4; H, 5·7%). Nitrogen, sulphur, phosphorus, and halogens were absent. Solutions of protoaphin-*ph* in concentrated sulphuric acid displayed no fluorescence and had no marked visible spectrum. Treatment of a solution of protoaphin-*ph* at pH 6 (N/100-sodium hydroxide adjusted with N/100-hydrochloric acid) with fresh crushed *A. philadelphi* gave xanthoaphin-*ph* (spectrum).

In earlier experiments, crystalline protoaphin-*ph* was obtained from the aqueous extract after removal of the methanol by rapidly treating it with just sufficient charcoal to lower the pH to 5, or by deionising a similar solution with ion-exchange resins.

The Aphins-sm. Living *A. sambuci* (100 g.) from elders were removed from the plants and worked up as described for the aphins-*fb* (Part II, *loc. cit.*). *Xanthoaphin*-sm (19·8 mg.), after two recrystallisations from ether–carbon tetrachloride, formed bright yellow crystals (Found: C, 63·1; H, 5·3. $C_{30}H_{30}O_{11}$ requires C, 63·6; H, 5·3%). Light absorption in chloroform: Maxima at 2590, 2810, 3590, 3790, 4070, 4310, and 4600 A.; $E^{1\%}_{1\,cm.}$ = 850, 900, 195, 387, 111, 184, and 196, respectively. *Chrysoaphin*-sm (100·4 mg.), after two recrystallisations from chloroform–carbon tetrachloride, was obtained as minute orange crystals (Found: C, 67·1; H, 5·4. $C_{30}H_{26}O_9$ requires C, 67·9; H, 5·0%). Light absorption in chloroform: Maxima at 2670, 3265, 3810, 4010, 4310, 4565, and 4855 A.; $E^{1\%}_{1\,cm.}$ = 720. 103. 398, 540, 110, 202, and 250, respectively. *Erythroaphin*-sm (182·4 mg.), after two

recrystallisations from chloroform–ethanol, formed dark-red needles (Found: C, 70·6; H, 4·8. $C_{30}H_{24}O_8$ requires C, 70·3; H, 4·7%). Visible light absorption in chloroform: Maxima at 4215, 4470, 4875, 5215, 5615, and 5865 A.; $E^{1\%}_{1\,cm.}$ = 532, 692, 105, 232, 356, and 148, respectively.

The Aphins-ce. *M. cerasi*, obtained from leaves of the wild cherry, were treated in the same manner as *T. salignus* (Part III, *loc. cit.*) to yield chrysoaphin-*ce* as a micro-crystalline orange powder (Found: C, 68·6; H, 4·7%). Light absorption in chloroform: Maxima at 2670, 3265, 3810, 4030, 4300, 4560, and 4850 A.; $E^{1\%}_{1\,cm.}$ = 735, 104, 398, 522, 122, 215, and 248, respectively. Erythroaphin-*ce* was obtained as dark-red needles (from chloroform-ethanol) (Found: C, 70·6, 70·6; H, 4·2, 4·2%). Visible light absorption in chloroform: Maxima at 4210, 4470, 4850, 5210, 5610, and 5870 A. $E^{1\%}_{1\,cm.}$ = 525, 684, 108, 228, 352, and 146, respectively. Xanthoaphin-*ce* was detected spectroscopically.

Oxidation of erythroaphin-ce *with nitric acid.* Erythroaphin-*ce* (100 mg.) was oxidised with nitric acid (3 c.c.) in the manner described for erythroaphin-*sl* (Part III, *loc. cit.*). The reaction product was treated exactly as before, and after methylation with diazomethane was purified by chromatography on neutral alumina. The combined crystalline fractions (29·7 mg.), obtained from the washings up to 50% light petroleum–benzene, were re-chromatographed on neutral alumina (0·84 g.) in order to separate further quantities of oily material. The crystalline fractions (11·5 mg.) were twice recrystallised from aqueous methanol, giving silky needles of hexamethyl mellitate, m. p. 185° undepressed in admixture with an authentic specimen, m. p. 187°.

The Aphins-ln. *E. lanigerum* ("woolly aphids" or "American blight"), as obtained from apple trees, is copiously surrounded with very waxy threads which were largely removed by a winnowing process by placing the insects in a fine gauze and gently rocking them to and fro while a moderate stream of air was applied from below. The aphins were then extracted by the method described previously for *A. fabæ*. Xanthoaphin-*ln* was not isolated but was detected spectroscopically, and the chrysoaphin-*ln* (lanigerin; Blount, *loc. cit.*) converted into erythroaphin-*ln* which formed long thin red needles (Found: C, 70·3; H, 5·1%); a chloroform solution of this erythroaphin showed the same spectrum as erythroaphin-*fb* in the comparison spectroscope.

The Aphins-py. Fresh *S. pyri* (48 g.), collected from leaves of cultivated pear trees, when treated as above, gave *xanthoaphin*-py (48 mg.), after two recrystallisations from ether–carbon tetrachloride, as a bright-yellow solid (Found: C, 60·7; H, 4·6. $C_{29}H_{30}O_{12}$ requires C, 61·1; H, 5·3. $C_{30}H_{32}O_{12}$ requires C, 61·6; H, 5·5%), together with *erythroaphin*-py as dark-red needles (51 mg.), after three recrystallisations from chloroform–ethanol (Found: C, 70·4; H, 4·6%). The light absorption of xanthoaphin-*py* in chloroform showed maxima at 2600, 2810, 3780, 4050, 4310, and 4570 A.; $E^{1\%}_{1\,cm.}$ = 860, 904, 320, 101, 180, and 181, respectively with an inflection at 3600 A.; $E^{1\%}_{1\,cm.}$ = 165. Visible light absorption of erythroaphin-*py* in chloroform: Maxima at 4220, 4480, 4850, 5220, 5610, and 5870 A.; $E^{1\%}_{1\,cm.}$ = 538, 720, 103, 230, 355, and 143, respectively.

Strobinin; *erythroaphin*-st. Strobinin (kindly provided by Dr. B. K. Blount) was recrystallised from chloroform–ethanol. Visible absorption spectra. (*a*) In chloroform: Maxima at 4200, 4470 5200, 5610, and 5860 A.; $E^{1\%}_{1\,cm.}$ = 541, 737, 243, 368, and 149. (*b*) In concentrated sulphuric acid: Maxima at 4580, 5260, and 5670 A.; $E^{1\%}_{1\,cm.}$ = 635, 432, and 800, respectively.

Grateful acknowledgment is made to the Council of Scientific and Industrial Research, Australia, for grants (to H. D. and J. P. E. H.) and to the Wellcome Trustees for a Fellowship (held by S. F. M.). The participation of one of us (S. F. M.) was made possible by Prof. C. H. Best, F.R.S., who gave facilities for carrying out work during 1947—1948 at the Department of Medical Research, University of Toronto. We are deeply indebted to Mr. H. L. G. Stroyan who has identified the Aphididæ and who also gave valuable assistance in the location of several of the species. Finally we record our thanks to Dr. B. K. Blount for a sample of strobinin and to Dr. R. N. Haszeldine for the absorption data.

UNIVERSITY CHEMICAL LABORATORY, CAMBRIDGE. [*Received, August 30th*, 1950.]