586. *Colouring Matters of the Aphididæ. Part V.* Infra-red Spectra.*

By A. W. Johnson, J. R. Quayle, T. S. Robinson, N. Sheppard, and A. R. Todd.

A detailed study of the infra-red spectra of the various aphins has been made; the erythroaphins from *Aphis fabæ*, *A. sambuci*, *Eriosoma lanigerum*, and *Myzus cerasi* have been found to give identical spectra, but those from *Tuberolachnus salignus* and *Sappaphis pyri* are each different from all the others so far examined. The spectra suggest that in all the erythroaphins the hydroxy-groups are strongly bonded with the quinone-carbonyl groups, *i.e.*, that the hydroxy-groups occupy *peri*-positions with respect to the quinone. Examination of the infra-red spectrum of diacetylerythroaphin further suggests that the quinone system in the erythroaphins is extended, *i.e.*, that the carbonyl groups are in different rings. No frequencies corresponding to non-bonded hydroxy- or carbonyl groups are present in the erythroaphin spectra, although both xanthoaphin and chrysoaphin appear to contain normal hydroxy-groups. Pyrolysis of erythroaphin at 230° gives acetic acid.

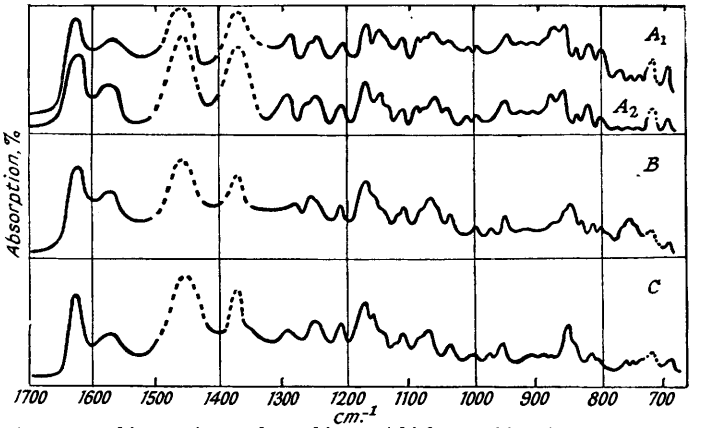
The earlier papers of this series (Duewell, Human, Johnson, MacDonald, and Todd, *Nature*, 1948, **162**, 759; *J.*, 1950, 477, 485, 3304) have described the inter-relationships of the aphin colouring matters, isolated from a number of aphid species. Each series comprises a protoaphin which is the non-fluorescent pigment of the living insects, and a xanthoaphin, chrysoaphin, and erythroaphin, the last three fluorescent pigments being obtained progressively from protoaphin by enzymic action and acid treatment. The question whether there are several aphid species which yield identical aphins is one which has important implications, biological as well as chemical, and we have referred to this point in our previous publications. Up to the present, the species of origin has been indicated by the addition of a two-letter suffix to the name of the particular colouring matter, *e.g.*, erythroaphin-*fb* (from *Aphis fabæ*) or -*sl* (from *Tuberolachnus salignus*), and so on (Part I, *loc. cit.*). Obviously, the question of nomenclature would be simplified, as well as the problems of collection of raw material, if the pigments from all the various species had not always to be kept separate.

The experimental evidence described in this paper suggests that the pigments from *A. fabæ* (host: beans, etc.), *A. sambuci* (elders), *Eriosoma lanigerum* (apple trees), and *Myzus cerasi* (cherry trees) are almost certainly identical, but that those from *T. salignus* (willow trees) and *Sappaphis pyri* (pear trees) are each unique. Information on the aphins from other species will be presented as it becomes available. It seemed probable from the evidence presented in Parts II and III that the aphins from *A. fabæ* were not identical with those from *T. salignus*, although they were undoubtedly very similar. The corresponding aphins of the two series were indistinguishable on the basis of visible and ultra-violet spectra and no means are known to us at present for the separation of mixtures of the corresponding pigments, *e.g.*, the erythroaphins-*fb* and -*sl*. Although erythroaphin-*fb* and erythroaphin-*sl* had the same or very similar molecular formulæ as far as could be judged from elementary analyses, and the same applied to the two chrysoaphins, the carbon and hydrogen figures for xanthoaphin-*fb* and xanthoaphin-*sl* differed considerably. Repetition of the isolation and analyses of the xanthoaphins has demonstrated that these compounds retain traces of solvents very tenaciously and, as they

* Part IV, *J.*, 1950, 3304.

are unstable to heat, the problem of solvent removal is more difficult than usual. The figures obtained for the carbon content of xanthoaphin-*sl* in the present work are much closer to those of xanthoaphin-*fb* previously reported, although consistent analyses on different batches of xanthoaphin-*sl* have still not been obtained. The retention of solvent seems to offer the most logical explanation of this phenomenon but there still remains the possibility that xanthoaphin

FIG. 1.

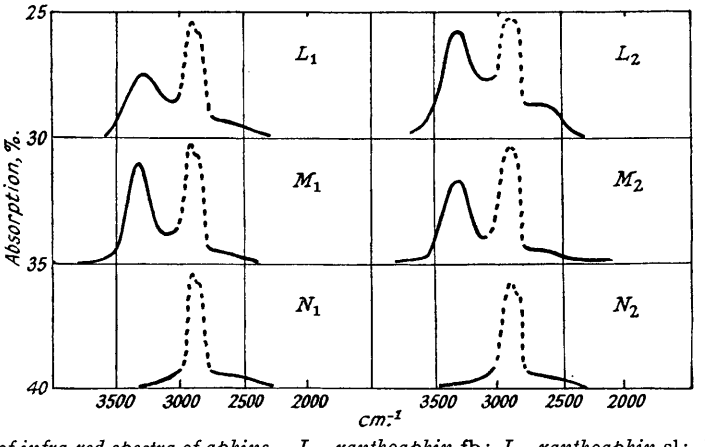


Infra-red spectra of erythroaphins. A_1, *erythroaphin-*sm (*thick sample*); A_2, *erythroaphin-*fb (*thin sample*); *B, erythroaphin-*sl; *C, erythroaphin-*py. *The spectra of erythroaphins-*ln *and* -ce *are identical with* A_1 *and* A_2.

may contain varying amounts of an undetected impurity. A formula, $C_{30}H_{28}O_{12}$, is favoured at present for xanthoaphin-*sl*, compared with $C_{30}H_{30}O_{11}$ for xanthoaphin-*fb*, but it is emphasised that these provisional molecular formulæ may well have to be amended later.

Small but significant differences between the aphin-*fb* series and the *sl* series were apparent in their solubilities, their behaviour on heating (the *sl* series decomposed on melting), their crystalline habits, and in the yields of mellitic acid obtained from the nitric acid oxidation of

FIG. 2.



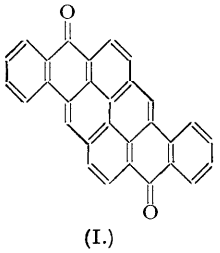
Hydroxyl region of infra-red spectra of aphins. L_1, *xanthoaphin-*fb; L_2, *xanthoaphin-*sl; M_1, *chrysoaphin-*fb; M_2, *chrysoaphin-*sl; N_1, *erythroaphin-*fb; N_2, *erythroaphin-*sl.

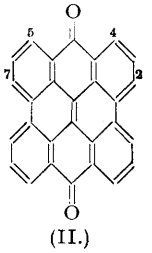
the erythroaphins, that from the *fb* pigment (and *ce*, Part IV, *loc. cit.*) being appreciably smaller than that from erythroaphin-*sl*. A study of the infra-red spectra of a variety of aphin pigments from different species has confirmed the difference between the *fb* and *sl* series and at the same time has shed a little more light on the structures of the pigments.

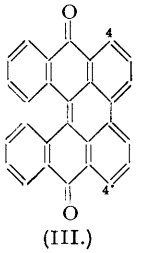
The infra-red spectra of the erythroaphins-*fb*, -*sm*, -*ln*, -*ce*, -*sl*, and -*py* (Figs. 1 and 2) were determined and a general survey of the spectra leaves no doubt as to the close relationship

between these compounds. Well-defined maxima are observed near 1625, 1575, 1290, 1260, 1210, 1170, 1115, 1070, 955, 855, and 820 cm.$^{-1}$ in all of the spectra. The four erythroaphins-*fb*, -*sm*, -*ln*, and -*ce* give spectra which are identical in every detail, and the first two of these are shown in Fig. 1. It therefore seems reasonable to deduce that they are identical substances. The spectra of the erythroaphins-*sl* and -*py* differ from the others in several details and in particular they have an extra distinct absorption at 975 cm.$^{-1}$ and lack a strong absorption at 880 cm.$^{-1}$. Only erythroaphin-*sl* shows a marked absorption band at 760 cm.$^{-1}$. These erythroaphin spectra were in the first instance considered in relation to the chemical evidence, cited in Parts II and III (*locc. cit.*) for the presence of quinonoid carbonyl groups and two phenolic hydroxyl groups in the erythroaphin-*fb* and -*sl* molecules.

The quinonoid grouping in the molecule would be expected to give rise to a strong absorption band corresponding to the stretching mode of the carbonyl group. The carbonyl frequencies of *p*-benzoquinone and anthraquinone have been given by Flett (*J.*, 1948, 1441) as 1660 and 1676 cm.$^{-1}$ respectively. As part of this investigation we have also observed the corresponding absorption band of 1 : 4-naphthaquinone at 1664 cm.$^{-1}$, and it seems from this limited evidence that simple *p*-quinones might be expected to absorb in the approximate region 1660—1680 cm.$^{-1}$. Josien and Fuson (*J. Amer. Chem. Soc.*, 1951, **73**, 478) have observed the carbonyl absorption bands of the extended quinones, 3 : 8- and 3 : 10-pyrenequinone, at 6·1 μ or approximately 1640 cm.$^{-1}$, and a further series of extended quinones has been studied spectroscopically in this laboratory (Hadzi and Sheppard, *J. Amer. Chem. Soc.*, in the press). The values found for the carbonyl absorption bands were as follows, only the strong absorption band with the highest frequency in the range 1550—1750 cm.$^{-1}$ being quoted in each case : pyranthrone (I), 1655 cm.$^{-1}$; *meso*naphthadianthrone (II), 1649 cm.$^{-1}$; helianthrone (III), 1646 cm.$^{-1}$; 1 : 2 - 6 : 7-dibenzopyrene-3 : 8-quinone (IV), 1645 cm.$^{-1}$; dibenzanthrone (V), 1638 cm.$^{-1}$; and diphenoquinone (VI), 1626 cm.$^{-1}$. It can be seen that the majority of these extended quinones have their carbonyl absorptions in the range 1655—1635 cm.$^{-1}$, a single exception being diphenoquinone with the frequency of 1626 cm.$^{-1}$. However, because of the unusual structure and chemical reactivity of the latter molecule it cannot be regarded as typical.


(I.)


(II.)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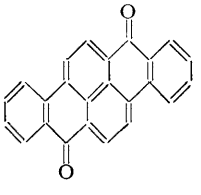
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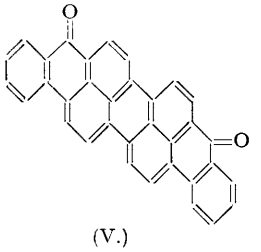
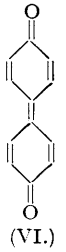

(V.)


(VI.)

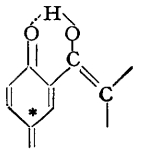
The observed value of the " carbonyl " frequency in the erythroaphin spectra is at 1625 cm.$^{-1}$, and it can be seen to be at the extreme lower end of the above range found for quinones and considerably lower than the frequencies found for the majority of simple or extended quinones. If this absorption band in the erythroaphin spectra is to be correlated with the presence of quinonoid carbonyl groups, it therefore appears that these are likely to be in unusual structural surroundings.

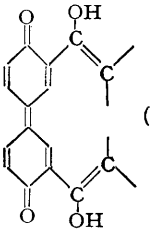
A second important feature of the erythroaphin spectra is apparent when consideration is given to the features to be expected from the presence of phenolic hydroxyl groups. Usually such groups give rise to strong and broad absorption bands near 3300 cm.$^{-1}$ which arise from

the stretching mode of the hydroxyl linkages, but none of the erythroaphin spectra has absorption bands in this region (see Fig. 2). However, there is now considerable evidence in the literature that both the carbonyl and the hydroxyl absorption bands are shifted markedly to lower frequencies when these two groups form chelated six-membered ring systems by means of hydrogen bonding (Gordy, *J. Chem. Physics*, 1940, **8**, 516; Flett, *loc. cit.*; Rasmussen, Tunnicliff, and Brattain, *J. Amer. Chem. Soc.*, 1949, **71**, 1068; Hinsberger, *ibid.*, 1950, **72**, 5626). Flett's work on the infra-red spectra of a series of hydroxyanthraquinones is particularly relevant and has shown that the presence of a β-hydroxy-group in the nucleus, where no intramolecular hydrogen bonding with the quinonoid carbonyl is possible, gives rise to normal carbonyl and hydroxyl frequencies near 1675 and 3350 cm.$^{-1}$ respectively. However, an α-hydroxy-group which can form a six-membered chelate ring with the quinone carbonyl invariably causes the appearance of a low carbonyl frequency in the range 1590—1640 cm.$^{-1}$, to be compared with 1676 cm.$^{-1}$ in the unsubstituted quinone, while the usual hydroxyl absorption band does not appear. The apparent lack of a hydroxyl absorption in the case of the α-hydroxyanthraquinones is almost certainly due to the fact that the very strong hydrogen bonding has caused the absorption band to occur at low frequencies in the OH absorption region near 3000 cm.$^{-1}$ in the form of a much broader band of lower peak intensity. We have re-investigated the spectra of 1-hydroxy-, 1 : 4-dihydroxy-, and 1 : 5-dihydroxy-anthraquinone originally studied by Flett (*loc. cit.*) and found a weak and broad absorption band centred near 2700 cm.$^{-1}$, in agreement with the above hypothesis. We have also made further observations on the infra-red spectra of extended quinones to include certain of the hydroxy-derivatives. Once again it was found that hydroxyl groups in α-positions caused lowering of the quinonoid carbonyl frequencies, and that when only such α-hydroxyl groups were present the *ca.* 3300-cm.$^{-1}$ absorption band could not be detected. The effect on the carbonyl frequency is well illustrated by the positions of these absorption bands in the following derivatives of *meso*-naphthadianthrone, only the absorption band with the highest frequency in the range 1550—1750 cm.$^{-1}$ being quoted in each case as before: *meso*naphthadianthrone, 1649 cm.$^{-1}$; 4 : 4′-dihydroxy*meso*naphthadianthrone (Attree and Perkin, *J.*, 1931, 144), 1620 cm.$^{-1}$; 2 : 2′-dimethyl-4 : 4′ : 5 : 5′ : 7 : 7′-hexahydroxy*meso*naphthadianthrone (hypericin; Brockmann, von Falkenhausen, and Dorlas, *Naturwiss.*, 1950, **37**, 540), 1589 cm.$^{-1}$; and also in the case of helianthrone and its substitution products: helianthrone, 1646 cm.$^{-1}$; 4 : 4′-dihydroxy-helianthrone (Attree and Perkin, *loc. cit.*), 1607 cm.$^{-1}$.

From the above data it can be concluded that the observed carbonyl frequency of the erythroaphins at 1625 cm.$^{-1}$, while abnormally low in value for a simple or extended quinone group in isolation, would be consistent with the presence of such groups if they formed part of chelated six-membered rings caused by hydroxy-groups in neighbouring α-positions. Such a formulation would at the same time explain the absence of the usual hydroxyl stretching mode near 3300 cm.$^{-1}$, and as can be seen in Fig. 2 there is some spectroscopic evidence for a weak and very broad absorption band centred near 2700 cm.$^{-1}$ which would be expected to replace the former absorption under such conditions. Thus, the spectroscopic evidence strongly suggests the presence of two of the units (VII) in the erythroaphins, the substituents on C* depending on whether the quinone system is contained in one ring or is extended. When

(VII.)





(VIII.)

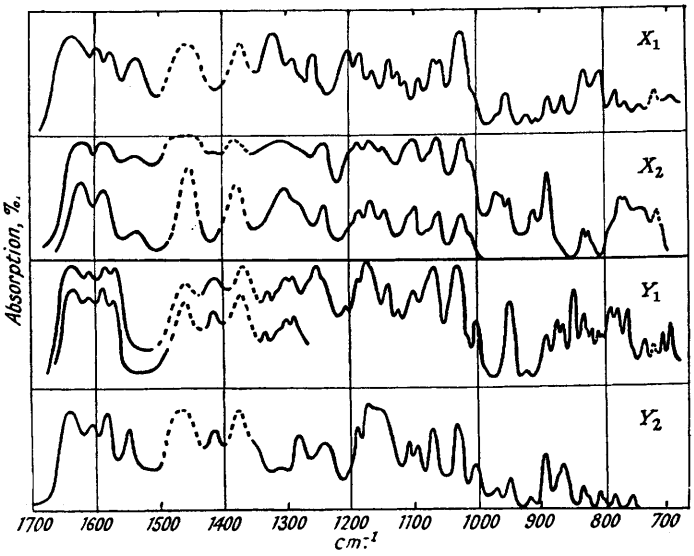
the hydroxy-groups of the hydrogen-bonded hydroxy-quinones are acetylated, the hydrogen bonding is destroyed and the position of the carbonyl absorption in the acetoxy-quinones reverts to a value comparable with that of the parent quinone (Flett, *loc. cit.*). In the case of erythroaphin-*sl*, the diacetyl derivative (Part III, *loc. cit.*) shows a carbonyl absorption band at 1637 cm.$^{-1}$ (in addition to an extra band near 1770 cm.$^{-1}$ due to the acetoxy-group) which strongly suggests the presence of an extended quinone system in erythroaphin-*sl*. Chemical evidence to support this deduction will be presented in a later paper. The available

infra-red data do not allow a choice of the type of extended quinone system present but the simplest expression of the facts so far presented would be the grouping (VIII).

Another property of the erythroaphins-*fb* and -*sl* can be considered in the light of the infra-red spectra. It has been found that at 230° in an atmosphere of nitrogen or *in vacuo*, the erythroaphins evolve 0·5—0·6 mole of acetic acid. Although at first we were inclined to interpret this result as indicating the presence of an inert acetoxy-group in erythroaphin (quantitative hydrolyses of diacetylerythroaphin and tetra-acetyldihydroerythroaphin indicate the presence of two and four acetyl groups respectively), the absence of evidence for any " normal " (*i.e.*, not hydrogen-bonded) carbonyl groups on the basis of the infra-red spectrum (a strong absorption band in the region of 1780—1750 cm.$^{-1}$ would be expected) renders this explanation improbable. The acetic acid must therefore arise from a more extensive thermal decomposition but the nature of the other products of the reaction has not yet been ascertained.

The infra-red spectra of the xanthoaphins-*fb* and -*sl* and of the corresponding chrysoaphins are shown in Figs. 2 and 3. They all show strong absorption bands in the region 1640—1620 cm.$^{-1}$ which can probably be correlated with the presence of carbonyl groups of extended

FIG. 3.



Infra-red spectra of xanthoaphins and chrysoaphins. X_1, *xanthoaphin*-fb; X_2, *xanthoaphin*-sl; Y_1, *chrysoaphin*-fb; Y_2, *chrysoaphin*-sl.

quinones. For xanthoaphin-*sl* the highest frequency in this region is, as for the erythroaphins, at 1625 cm.$^{-1}$, suggesting that in this case both the quinonoid carbonyls are chelated. In agreement with this hypothesis, xanthoaphin-*sl* has a particularly marked broad absorption centred near 2700 cm.$^{-1}$ attributable to the stretching frequency of chelated hydroxyl bonds. Xanthoaphin-*fb* also has an absorption band in this region and similar, but weaker, features seem also to be present in the chrysoaphin spectra. Unlike the erythroaphins, the xanthoaphins exhibit strong absorption bands between 3300 and 3400 cm.$^{-1}$ (Fig. 2) which provide good evidence for the presence of additional hydroxyl groups of the normal type such as occur in the spectra of alcohols and phenols. In the region 1600—700 cm.$^{-1}$ there are several strong absorption bands common to the spectra of the two chrysoaphins, *viz.*, near 1415, 1170, 1070, 1030, 1000, and 950 cm.$^{-1}$ which show that they are closely related substances. There are fewer coincidences between the spectra in this region for the xanthoaphins-*fb* and -*sl* and this is in agreement with the chemical evidence cited above that these two substances are of rather different structure. The spectra, however, still maintain a strong general resemblance.

EXPERIMENTAL.

Spectroscopic Methods.—The infra-red spectra of the aphins were run as mulls in Nujol prepared from the crystalline materials. The infra-red spectrometers used were of the Perkin–Elmer 12B and Hilger D209 types used with rock-salt prisms under standard conditions (Sheppard and Sutherland, *J.*, 1947, 1540).

Xanthoaphin-sl.—Prepared as described in Part III (*loc. cit.*) and crystallised, as before, from ether–carbon tetrachloride it had m. p. 210° (decomp.) (Found, in material dried at room temperature: C, 62·4, 62·45, 63·0, 63·7; H, 4·7, 4·8, 4·6, 4·7. $C_{30}H_{28}O_{12}$ requires C, 62·1; H, 4·6. $C_{30}H_{26}O_{12}$ requires C, 62·4; H, 4·5%). A further sample was crystallised from ether–benzene (Found, in material dried at room temperature: C, 67·7, 68·0; H, 5·25, 5·2. $C_{30}H_{28}O_{12},2C_6H_6$ requires C, 68·5; H, 5·5%).

Pyrolytic Decomposition of Erythroaphin.—Erythroaphin-*fb* (104 mg.) was introduced into a small hard-glass test-tube and a pad of purified asbestos placed over the pigment. The test-tube was sealed on to a length of glass tubing leading into a liquid-air trap and the whole evacuated and sealed. The pigment was then heated at 230° for 8 hours, after which the tube was opened and the distillate treated with piperazine hexahydrate. The resulting piperazine salt was repeatedly washed with ether, crystallised from *n*-butanol, sublimed, and then recrystallised as before; it had m. p. 204°, alone and mixed with an authentic specimen of piperazine acetate. An identical product was obtained from the pyrolysis of erythroaphin-*sl*. In further experiments the acetic acid so obtained was determined by direct titration with standard alkali. From erythroaphin-*fb* there was obtained 0·60 mole of acetic acid, and from erythroaphin-*sl* 0·54 mole.

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