## 9. *Colouring Matters of the Aphididæ. Part XVI.*[1] *Reconsideration of the Structure of the Erythroaphins.*

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Further investigation has shown that the erythoaphins contain only two " aromatic " CH groups and that they contain in addition two Me·CH·O– groupings. Previous structures tentatively assigned to these pigments are therefore untenable.

In 1948 we first reported the existence of a remarkable group of colouring matters to which we gave the general name " aphins." [2] These substances occur in the hæmolymph of many dark species of *Aphididæ* or are derived from substances present in the hæmolymph. For each insect species there are four aphins to be considered: (*a*) the protoaphin, a yellow glucosidic pigment present in the hæmolymph of the living insect; (*b*) the yellow xanthoaphin, which is sugar-free and is formed from protoaphin by a specific enzyme system present in the insect and bringing about the change on the death of the insect; (*c*) the orange chrysoaphin, produced when xanthoaphin is kept in solution or, more rapidly, by means of acid or alkali; and (*d*) the red erythroaphin, formed in a similar way from chrysoaphin and representing the relatively stable end-product of the series. The conversions (*b*) ⟶ (*c*) ⟶ (*d*) occur spontaneously in extracts of the insects and can be conveniently followed by spectroscopic observation. All the aphins so far studied in detail belong to one or other of two series, corresponding pigments in each series being apparently

[1] Part XV, Johnson, Todd, and Watkins, *J.*, 1956, 4091.
[2] Duewell, Human, Johnson, MacDonald, and Todd, *Nature*, 1948, **162**, 759.

stereoisomeric with one another. The two series we describe as the aphins-*fb* and the aphins-*sl*, these designations being derived from the name of the insect species from which they were first isolated—aphins-*fb* from the common bean aphid *Aphis fabae* and aphins-*sl* from the willow aphid *Tuberolachnus salignus*. There exist therefore a protoaphin-*fb* and a protoaphin-*sl* each the parent substance of a distinct series of pigments. In our earlier studies we examined several other aphid species in a preliminary way and some were shown to yield aphins-*fb*. In particular, however, erythroaphin-*py* from *Sappaphis pyri* appeared possibly different from both erythroaphins-*fb* and -*sl*.[3] This has not been substantiated by subsequent work [4] which suggests identity with the -*sl* isomer. The isolation of protoaphin-*ph* [5] from *A. philadelphi* has also been described, but, for lack of material, no further work on this series has been carried out and there are no grounds for suggesting that it differs from the -*fb* or -*sl* series. The pigments obtained from *Hamamelistes* species,[6] though probably closely related to the erythroaphins, are structurally different and are not considered further here.

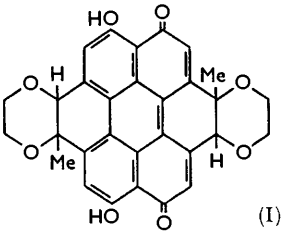
In endeavouring to elucidate the structure of the aphins and the nature of the observed interconversions, it was decided to concentrate attention first on the stable erythroaphins. An extended series of investigations led in 1955 to the advancement of two possible structures (I) and (II) for erythroaphin.[7] The difference between the -*fb* and the -*sl* isomers was attributed to the stereochemistry at the junction of the heterocyclic rings with the rest of the molecule,[8] it being considered that both rings were *cis*-fused in erythroaphin-*fb*, one *cis*- and the other *trans*-fused in the *sl*-isomer. Of the two possibilities, (I) was preferred since, although acetaldehyde was obtained from the erythroaphins by acid treatment, the conditions necessary for its formation were very much more vigorous than would have been expected on the basis of formula (II) which contains two dioxolan rings.

Neither structure could, however, be regarded as established and, indeed, although all the complex reactions of the erythroaphins could be explained on the basis of either, there were a number of detailed points for which they provided no very satisfactory explanation. The difficulty with which acetaldehyde could be produced was easier to explain on the basis of (I) than (II), but, on the other hand, all attempts to prepare derivatives of ethylene glycol from erythroaphin derivatives failed and oxidation by the Kuhn–Roth procedure always gave *ca.* 3·8 mol. of acetic acid, results more in keeping with structure (II) than (I). Again, on either formula the amount of coronene, as compared with that of perylene, derivatives produced on zinc dust distillation [9] was surprisingly low. Finally, the visible and ultraviolet spectra of tetra-acetyldihydroerythroaphin, although almost identical in general pattern with that of 3,4,9,10-tetra-acetoxyperylene, showed a bathochromic shift of nearly 50 mμ relative to the latter; [10] a shift of this magnitude is difficult to reconcile with a structure in which there are no oxygen atoms attached directly to the perylene system other than in positions 3, 4, 9, and 10.

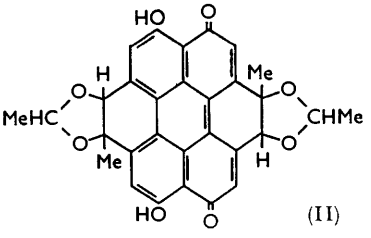
Further difficulties arose when a closer study of the other aphin pigments was undertaken. In our earlier work we were able to give only tentative molecular formulæ for these and, not unexpectedly, closer examination with larger amounts of material showed that revision was necessary. As will be reported in the following paper, protoaphin has a formula $C_{36}H_{38}O_{16}$, xanthoaphin $C_{30}H_{26}O_{10}$, and chrysoaphin $C_{30}H_{24}O_9$. The formation of xanthoaphin from protoaphin is thus accompanied formally by the loss of 1 mol. of glucose; xanthoaphin loses 1 mol. of water in passing into chrysoaphin; and chrysoaphin yields erythroaphin $C_{30}H_{22}O_8$ again by loss of 1 mol. of water. Although it was possible

[3] Johnson, Quayle, Robinson, Sheppard, and Todd, *J.*, 1951, 2633.
[4] Calderbank, Ph.D. Thesis, Cambridge, 1954.
[5] Duewell, Human, Johnson, MacDonald, and Todd, *J.*, 1950, 3304.
[6] MacDonald, *J.*, 1954, 2378.
[7] Brown, Calderbank, Johnson, Joshi, Quayle, and Todd, *J.*, 1955, 959.
[8] Brown, Calderbank, Johnson, Quayle, and Todd, *J.*, 1955, 1144.
[9] Brown, Johnson, Quayle, and Todd, *J.*, 1954, 107.
[10] Calderbank, Johnson, and Todd, *J.*, 1954, 1285.

on the basis of formula (I) or (II) to formulate more or less plausible structures for xanthoaphin and chrysoaphin, the derivation of a compatible structure for protoaphin, known at the time to be naphthalenic, was well-nigh impossible.




(I)




(II)

Reconsideration of the evidence on which formulæ (I) and (II) were based was clearly necessary. The erythroaphins are undoubtedly derivatives of 4,9-dihydroxyperylene-3,10-quinone in which the chromophoric system is substituted by two symmetrically disposed non-aromatic groupings each containing five carbon and two ethereal-oxygen atoms. The erythroaphins react readily with amines to give diamino-derivatives in a manner apparently analogous to the well-known amination of quinones (including the perylenequinones). They can also be halogenated to dihalogenoerythroaphins which can react further with amines under mild conditions to give diaminodihalogenoerythroaphins.[11,12] The properties of all these erythroaphin derivatives are consistent with the substituents' having been introduced directly on the perylene nucleus, and in our earlier work these facts were taken as evidence that in erythroaphin itself there must be four substitutable " aromatic " CH groups. This has always been recognised by us to be the basic assumption in the arguments used in deriving structures (I) and (II); indeed, if it is accepted, (I) and (II) are the only possible formulations for erythroaphin. But it was merely an assumption and in taking up the problem afresh it was decided first to put it to the test by careful infrared and nuclear magnetic resonance spectroscopic studies. These established beyond doubt that the dihalogenoerythroaphins contain no aromatic CH group, *i.e.*, that there are only two free positions in the perylene nucleus of erythroaphin. Moreover, the nuclear magnetic resonance results indicated, not only that erythroaphin contains 4 side-methyl groups, but that each of these is attached to a carbon carrying both a hydrogen and an oxygen atom, *i.e.*, that the molecule contains 4 groupings of the type $CH_3-C(H)(-O-)$. Both these findings are incompatible with structures (I) and (II).

We have therefore carried out in the last few years a comprehensive attack on the aphin problem, studying simultaneously all four types of pigment, and we have arrived at a solution which explains all the facts rationally and without difficulty. These investigations are described in detail in the succeeding six papers (Parts XVII—XXII). As the researches involved are of some complexity these papers do not present a chronological account, but rather a selection of results set out to deal with the structure and the absolute stereochemistry of the protoaphins, erythroaphins, xanthoaphins, and chrysoaphins, in that order.

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[11] Brown, Johnson, MacDonald, Quayle, and Todd, *J.*, 1952, 4928.
[12] Brown, Calderbank, Johnson, MacDonald, Quayle, and Todd, *J.*, 1955, 954.