# The Chemical Basis of the Color Dimorphism of an Aphid, *Macrosiphum liriodendri* (Monell), and a Locust, *Amblycorypha* sp. Novel Carotenoids

It has long been known that certain aphid species occur in morphologically identical color variants (e.g. green and pink, etc.). This phenomenon is also found in other insects (e.g. grasshoppers). Since aphids reproduce parthenogenetically during the summer, and since colonies living side by side on the same leaf or stem, i.e. under identical environmental conditions, often include individuals of both color types, the occurrence of this dimorphism is quite surprising; its causes seem still obscure. As a contribution to this problem, we have investigated the chemical nature of the pigments of *Macrosiphum liriodendri* (Monell), a species which forms colonies of pink, and of yellowish to bluish green individuals on the underside of leaves of the tulip tree, *Liriodendron tulipifera*, in May and June. The pink form is sometimes referred to as var. *rufa* (Monell). The very similar color dimorphism of the closely related species, *M. euphorbiae* (formerly called *M. solanifolii*), which occurs on rose, potato, and other plants, has been studied in detail by SHULL[1], who observed that isolated parthenogenetic individuals yield offspring of their own color exclusively; offspring of fertilized eggs can consist of both green and pink individuals, even when both parents were of the same strain of either pink or green color. Crossing of green and pink parents seems to have given only green progeny.

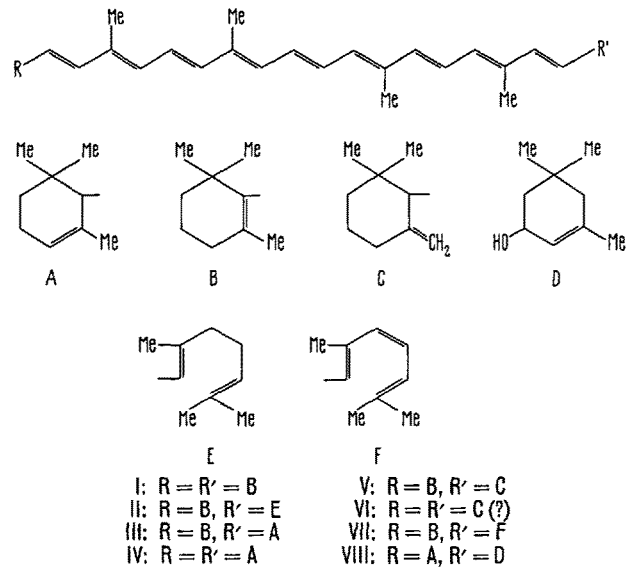
The color dimorphism of *M. liriodendri* has apparently not been studied in any detail, although the biology of this aphid has been examined by DAVIS[2]. For our purpose of chemical investigation, this shortcoming is more than offset by the abundant occurrence of the species in the immediate vicinity of our laboratory at NIH. We have observed the color of aphids produced either by winged stem-mothers, or by young colonies of uniform color, isolated with the help of gauze bags on leaves of the host plant, and have found that here, too, no change in pigmentation seems to take place in subsequent generations of parthenogenetic offspring.

For the chemical examination, collections of hand-picked green and pink individuals were extracted separately with acetone, and the filtered extracts were evaporated in vacuo. Extraction of the residues with hexane yielded filtrates of strikingly different color: greenish-yellow from the green, brownish-pink from the pink aphids. The pigments chiefly responsible for the coloration of the insects are thus remarkably non-polar; hence the dimorphism can not, as might have been assumed, be based upon differences in the well-known glycosidic aphid pigments investigated in the laboratory of Lord TODD[3], e.g. occurrence of a dactynaphin in the pink, the related aphinin in the green variety. The hexane-insoluble parts of the acetone-extracts, and the acetone-extracted aphid bodies, both contained a deep green pigment, extractable with acetic acid; from its spectrum this pigment seems to be identical with, or closely related to, the aphinin studied by CAMERON et al.[3b].

The absorption spectra of both hexane extracts suggested that the pigments are carotenoids. Since chromatographic tests showed that within limits of detection, the red pigments responsible for the color of hexane extracts from the pink specimens are entirely absent from those of the green ones, the very laborious manual separation of the color variants was considered unnecessary, and mixed batches of aphids were used for subsequent closer study. The hexane-soluble fractions were saponified with 5% methanolic potassium hydroxide for 12 h at room-temperature, and the pigments were separated on circular paper chromatograms (Schleicher and Schüll No. 288), using 2% acetone in hexane as solvent. In order of increasing polarity, a yellow zone containing fractions 1–4, and 3 pink zones (5–7) were obtained. The 4 yellow to orange pigments of the yellow zone were separated by column chromatography on neutral alumina (act. grade II) with hexane as eluant.

Fractions 1–4 all had the same mass-spectroscopic molecular weight of 536, showing them to be carotenes, $C_{40}H_{56}$. Fractions 1 and 4 were identical, spectroscopically and chromatographically, with $\beta$-carotene (I) and $\gamma$-carotene (II), respectively. Fractions 2 and 3 agreed spectroscopically with $\alpha$-carotene (III) and $\varepsilon$-carotene (IV), respectively, but differed from these by lower Rf values on paper chromatograms. Fraction 2 was found to be identical with a carotene, provisionally named P-444, which has recently been isolated from the discomycete, *Caloscypha fulgens* (Pers.) Boud., and identified as V[4]. It is the first natural carotenoid to contain the $\gamma$-ionone ring (C).

Fraction 3, spectroscopically identical with $\varepsilon$-carotene (IV), is again more strongly adsorbed, and since this property seems to be typical for carotenes containing the $\gamma$-ionone ring[4], the compound may well be the hitherto unknown carotene with 2 $\gamma$-ionone rings (VI)[5]. Structure VI has now been unequivocally established (S. LIAAEN-JENSEN and A. G. ANDREWES, private communication). Chromatographic studies with the earlier extracts from the separate batches of green and pink specimens suggest that V and VI occur only in the former, II only in the latter variant; $\beta$-carotene seems to be present in both. However, scarcity




I: R = R′ = B
II: R = B, R′ = E
III: R = B, R′ = A
IV: R = R′ = A
V: R = B, R′ = C
VI: R = R′ = C (?)
VII: R = B, R′ = F
VIII: R = A, R′ = D

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[2] J. J. DAVIS, Ann. ent. Soc. Am. *2*, 36 (1909).
[3] a) LORD TODD, Chem. Brit. *2*, 428 (1966); b) J. H. BOWIE, D. W. CAMERON, J. A. FINDLAY and J. A. K. QUARTEY, Nature, Lond. *210*, 395 (1966).
[4] N. ARPIN, J.-L. FIASSON, M. P. DANGYE-CAYE, G. W. FRANCIS and S. LIAAEN-JENSEN, Phytochem., in press (1971).
[5] K. H. WEISGRABER, R. J. J. CH. LOUSBERG, U. WEISS and S. LIAAEN-JENSEN, to be published.

of material made conclusive proof of this distribution impossible.

The pink carotenoids, fractions 5, 6, and 7, gave mass-spectrometric molecular weights of 534. The absorption spectrum of the first of these suggests a dodecaene; the one of fraction 6 is identical with that of torulene, (VII), from the yeast *Rhodotorula*. Identity seems probable. Carotenoid 7 could not be investigated in detail, since its pink color tends to disappear rapidly. Its absorption spectrum indicates a *cis*-tridecaene chromophore. Details of the chemical study of the pigments will be published elsewhere[5].

These results show that the color dimorphism of *M. liriodendri* is entirely due to differences in the carotenoids, and that these carotenoids, except for the ubiquitous $\beta$-carotene (I), and for $\gamma$-carotene (II), belong to uncommon types observed previously only in certain microorganisms. This unexpected finding strongly suggests that the color dimorphism of at least this one species of aphid may be based on differences in the symbiotic microorganisms present in all aphid species studied so far in this respect[6], (unfortunately, this does not include *M. liriodendri*), rather than on differences in the aphids themselves. The question as to the origin of these differences remains unanswered, but it may be significant that 2, sometimes even 3, morphologically different types of symbionts have been observed in certain aphid species. Apparently, it has never yet been possible to isolate the symbiotic microorganisms from aphids, or to cultivate them on artificial media, so that their taxonomic position is unknown; our results may perhaps give a hint in this direction. The inherited nature of the color type, mentioned before, is entirely compatible with our hypothesis, since elaborate mechanisms exist in aphids for the transmission of the microsymbionts, both to the eggs produced in autumn and to the embryos of the successive viviparous parthenogenetic generations in the warm season (ref.[6] pages 309, and 315, 321, respectively).

From the findings on the distribution of the yellow and red pigments in the 2 variants, one may tentatively conclude that the green form produces carotenes with the novel $\gamma$-ionone ring (V, VI), while the pink form seems to elaborate pigments with aliphatic structures on one end (II, VII).

A very similar color dimorphism is observed in certain katydids of the genus *Amblycorypha* (Orthoptera, Tettigoniidae). These insects are normally green but occur also in extremely rare pink forms. (A very good color picture of both a pink and a green specimen of *A. rotundifolia* is shown on the cover of the January 1966 issue of Biosciences, Vol. *16*, 1). Through the kindness of Prof. T. J. WALKER, University of Florida, Gainesville, Fla., we obtained 1 nymph of the pink form of *Amblycorypha* sp. (probably *A. floridana* Rehn and Hebard; positive identification is possible in the adult only), and have made some observations on the chemical nature of its pink pigments; our results are presented here, in spite of the very preliminary nature of the information obtainable from study of one single, fairly small specimen, because very little is known on the phenomenon of color dimorphism, and because the rarity of the pink variant leaves little hope for further work.

Attempts to extract the pink pigment from the exoskeleton by grinding with methanol or acetone invariably led to instantaneous disappearance of the pink color. However, extraction with water readily gave a bluish-pink, slightly opalescent solution; its spectrum consisted of 4 bands at $\lambda_{max}$ 457, 484, 524 and 567 nm, the last one being the most intense. The color can thus not be due to free carotenoids as in the case of *Macrosiphum*, in spite of the great resemblance of the visible pigmentation of the pink variants of both insects.

The pink color of the solution turned to light yellow at once on addition of methanol or acetone, or on attempted chromatography over celite. This behavior is quite analogous to that of the carotenoproteins which have been observed repeatedly in arthropods[7], including some grasshoppers[8]. The yellow pigment formed on filtration through a celite column (chromatographically one major and several very minor individuals) is soluble in hexane; the spectrum of the hexane solution is that of an $\alpha$-carotene, and identity with $\alpha$-cryptoxanthin (VIII) seems possible on spectroscopic and chromatographic grounds. No further experimental work was possible, but the results show that the pink color is due to a carotenoid complex, and the analogy with the behavior of known carotenoproteins[7] suggests that here, too, a protein complex is present.

The few carotenoproteins studied in detail so far[7] have orange to red ketonic carotenoids (canthaxanthin or astaxanthin) as the prosthetic group; the former in the red ovorubin from a gastropod, the latter in the green ovoverdin from lobster eggs and the blue crustacyanin from lobster carapace. The spectrum of the yellow carotenoid fraction from *Amblycorypha* is definitely not that of a ketonic carotenoid. It seems widely assumed[7] that the carbonyl group is involved in the binding of the carotenoid to the protein, and in the resulting spectroscopic shifts. In the present case, this explanation for the formation of a pink (protein ?) complex from a yellow carotenoid cannot apply, and the bathochromic effect must have some other cause.

*Zusammenfassung.* Der Unterschied in der Färbung von rosa und grünen Varianten der Blattlaus *Macrosiphum liriodendri* beruht auf Unterschieden der Carotinoid-Fraktionen. Einige dieser Carotinoide gehören zu Typen, die bisher nur in Mikroorganismen angetroffen worden sind, was Verschiedenheit in Art oder biosynthetischer Aktivität von Mikrosymbionten als Ursache des Pigmentierungs-Dimorphismus vermuten lässt. Die Färbung der seltenen rosa Variante der Heuschrecke *Amblycorypha* scheint durch einen Carotinoid-Protein-Komplex bedingt zu sein.

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[9] The authors are indebted to Dr. S. LIAAEN-JENSEN for identification of our carotenoids, advice on chromatographic techniques, and advance information on her pigment P-444, to Dr. LOUISE M. RUSSELL for taxonomic identification of the aphid species, to Dr. D. W. CAMERON for that of the aphinin, and to Mr. W. E. COMSTOCK for mass spectra.

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