

Animal Carotenoids

7.* Carotenes of Two Colour Variants of the Aphid *Macrosiphum liriiodendri* — Identification of Natural γ , γ -Carotene

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The carotenoid composition of the aphid *Macrosiphum liriiodendri* Monell has been studied as a joint project in our two laboratories. This aphid occurs in May and June in green and pink colour variants on the leaves of the tulip tree, *Liriodendron tulipifera* L., on the reservation of the National Institutes of Health in Bethesda. Aphids have been collected and examined in four successive seasons and we now present the results of this study. Some preliminary data have been reported and the reason for the colour dimorphism observed in this aphid has been discussed.¹

The best isolation procedure involved acetone extraction, removal of white contaminants by fractional crystallization from acetone, saponification by the normal procedure² to remove lipids, and TLC on alumina plates for individual characterization of the carotenoids. Co-chromatography tests with authentic carotenes were carried out on circular alumina-containing paper.³ Hand-picked pure batches of green and red aphids as well as mixed batches (for larger quantity of pigment) were examined. One gram of each colour variant (dry weight) provided 0.12 mg carotenoids; a mixed batch of 50 g (wet weight) being the

maximum available. All carotenoids encountered were carotenes. The new IUPAC nomenclature is used here.⁴ The γ -prefix is used⁵ to denote the cyclic end group characteristic of γ -ionone.

Green aphids. The carotenoid composition of green aphids is given in Table 1 and Scheme 1. Table 1 also includes R_F -values and spectral characteristics in visible light.

β,β -Carotene (1) was identified from its electronic spectrum, mass spectrum (m/e 536 = M, M-92, M-106⁶) and co-chromatography test with synthetic β,β -carotene.

The major pigment, β,γ -carotene (2), exhibited an electronic spectrum identical with that of β,ϵ -carotene (α -carotene by old nomenclature), from which it differed in chromatographic behaviour. The order of increasing adsorbance on alumina paper was β,ϵ -carotene (from carrots), β,β -carotene (1), and β,γ -carotene (2).

The identification was based on co-chromatography with natural⁵ and synthetic⁷ β,γ -carotene (2), and on mass spectrometry. The aphid pigment exhibited the molecular ion at m/e 536 and a fragmentation pattern analogous to that reported for natural and synthetic β,γ -carotene (2). This is the second finding of β,γ -carotene (2) in nature. 2 was first isolated from the discomycete *Caloscypha fulgens* (Pers.) Boud.⁵

The third pigment had absorption characteristics in visible light typical of an aliphatic nonaene chromophore. On alumina paper this pigment was less strongly adsorbed than the aliphatic carotene neurosporene (from *Rhodospseudomonas gelatinosa* (Molisch) van Niel⁸), but more strongly adsorbed than synthetic⁹ ϵ,ϵ -carotene and β,γ -carotene (2). The hypothesis of this pigment being a new carotene (3) with two γ -rings was confirmed by direct comparison with a synthetic⁷ specimen. The mass spectrum of the natural pigment further showed the expected molecular ion at m/e 536 and the loss of 92, 106, and 158 mass units.^{6,10} This is the first identification of γ,γ -carotene (3) in a natural source.

Pink aphids. The carotenoid composition of the pink aphids was strikingly different from that of the green variety. Three major and two minor pigments were isolated (Table 1). β,β -Carotene (1) was identified on the basis of the criteria described above. The monocyclic β,ψ -carotene (4, γ -carotene by old nomenclature) was identified on the basis of identical electronic spectra, co-chromatography test with synthetic¹¹ β,ψ -carotene, and from its mass spectrum (m/e 536 = M, M-69, M-92, M-106, cf. Ref. 6).

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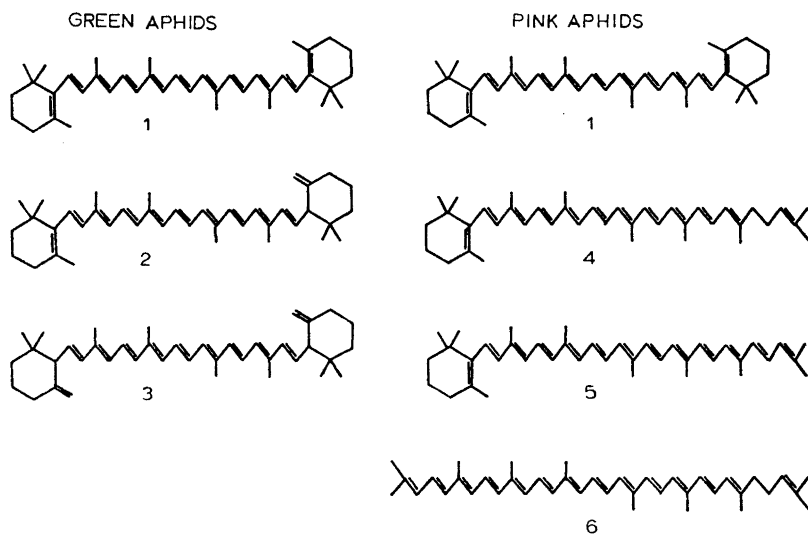
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Table 1. Carotenes present in the green and pink variety of *Macrosiphum liriodendri*.

Carotene	R_F -value *	λ_{\max} petroleum ether	Relative % **
Green aphids			
β,β -Carotene (1)	0.30 ^a	(425), 449, 475	10
β,γ -Carotene (2)	0.20 ^a	421, 443, 472	69
γ,γ -Carotene (3)	0.16 ^a	414, 439, 469	21
Pink aphids			
β,β -Carotene (1)	0.30 ^a	(425), 449, 475	10
β,ψ -Carotene (4)	0.56 ^b	436, 461, 490	10
Torulene (5)	0.24 ^b	458, 481, 513	80
3,4-Didehydro- ψ,ψ -carotene (6)		365, 385, 465, 492, 527	
Lycopene		365, (445), 470, 500	

* Schleicher & Schüll No. 288 (Al₂O₃) paper. ^a Petroleum ether. ^b 5 % ether in petroleum ether.

** Analysis performed in 1971.



Scheme 1.

The main carotenoid of the pink aphids was identified as torulene (5). The molecular weight (534) was determined by mass spectrometry; the electronic spectrum agreed with that obtained for synthetic¹² torulene. Similar R_F -values were obtained in separate experiments, but a direct comparison could not be carried out.

Synthetic torulene (λ_{\max} 454, 481, and 514 nm in petroleum ether, $R_F=0.24$) provided on iodine-catalyzed isomerization in benzene a neo A isomer (24 % of total; λ_{\max} 378, (458), 478, and 511 nm, $R_F=0.29$); a neo B isomer (12 % of total; λ_{\max} 378, (455), 477, and 510 nm, $R_F=0.32$) and a neo C isomer (14 % of total; λ_{\max} 378, (455), 476, and 510 nm, $R_F=$

0.35), the two latter with low spectral fine-structure. R_f -values refer to alumina paper and 5 % ether in petroleum ether. Equivalent *cis* isomers were encountered for the natural carotene.

A minor pigment with electronic spectrum compatible with an aliphatic tridecaene chromophore and the molecular ion at m/e 534 exhibited chromatographic properties compatible with 3,4-didehydro- ψ,ψ -carotene (6, 3,4-dehydro-lycopene by old nomenclature) but was not rigorously identified.

In one isolation another minor pigment was encountered with electronic spectrum and chromatographic behaviour indicative of ψ,ψ -carotene (lycopene).

From a mixed batch (green and pink aphids) a minor carotenoid with nonaene-type electronic spectrum inseparable from neurosporene was isolated.

Conclusion. The data obtained demonstrate the presence of bicyclic carotenes with β - and γ - (new nomenclature) end groups in the green aphids, and the absence of carotenes with γ -end groups in the pink variety. The pink variety contains bicyclic and monocyclic carotenes with β - and ψ -end groups, the ψ -end group has suffered further dehydrogenation.

It has been suggested that differences in the carotenoid composition might arise from different symbiotic microorganisms.¹

De novo carotenoid synthesis in animals is not commonly accepted,¹³ although the biosynthesis of β,β -carotene (I) by bovine *corpus luteum* tissue has recently been demonstrated.¹⁴ Whether the carotenes are produced by the aphids themselves or symbions cannot be decided from our experiments.

Irrespective of the site of *de novo* synthesis, it seems likely that the differences in carotenoid composition of green and pink aphids are caused by differences in the levels of cyclization enzymes. It has been demonstrated by others¹⁵ that independent biochemical reactions lead to ϵ - and β -rings. Thus the pink aphid may lack the enzyme necessary for the cyclization which yields the γ -ring and instead the process of dehydrogenation is continued. This interpretation is consistent with the observations that the total carotene content in both variants is the same and that both variants contain the same quantity of β,β -carotene (I).

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