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Plant Pigments identified in the Common Blue Butterfly

FLAVONOIDS are rare in the animal kingdom, but Ford¹, using a simple colour test, suggested that they were present in a number of butterflies. A difficulty in characterizing the pigments is the fact that a large number of specimens, of the order of hundreds, is required, and it is not always possible to obtain one species in such large numbers. Morris and Thomson², using some 400 specimens, identified tricetin and a glycoside of this flavone as well as a glycoside of another flavone, orientin, from the wings of the marble white butterflies *Melanargia galathea*. They found³ that the small heath butterflies *Coenonympha pamphilus* contained tricetin both free and as glycoside but not orientin. The flavonoids seem to be obtained by the insects through the caterpillars feeding on a particular food host. Carotenoids, on the other hand, are well known in animals, and Goodwin⁴ has suggested that insects accumulate their dietary carotenoids as such or that they may be altered to form other carotenoids not found in the insects' food. A number of specimens of the common blue butterfly *Polyommatus icarus* Rott. were collected both in France and in England with a view to characterizing the flavonoids and the carotenoids and to comparing these with their food plants. One hundred specimens of *P. icarus* from Box Hill, Surrey, were feeding on rest harrow *Ononis repens* and those from St Martial, France (800 specimens), on a species of *Anthyllis*; both plants are members of the Leguminosae.

Flavonoids were extracted by conventional methods⁵, refluxing with 70 per cent methanol for 2 h. The extract was evaporated *in vacuo* at about 40° and the residue dissolved in a small volume of 70 per cent methanol. This was then streaked on Whatman No. 1 paper and run in various solvents (Table 1). The characteristic colours under ultraviolet light with and without ammonia fumes were noted. *R_f* values and absorption spectra were compared with those given by Harborne⁶. Not all the bands that were obtained after chromatography in 30 per cent acetic acid were identified, because some were found in too small amounts. The compounds that were tentatively identified in the French butterflies were the free flavonol 3-O-methyl-kaempferol and the flavonol glycoside quercetin 3,4'-diglucoside (Table 1). A flavone glycoside and an isoflavone with properties shown in Table 1 were also obtained, but it was not possible to identify them further.

Table 1. FLAVONOIDS IN THE FRENCH COMMON BLUE

Band	Colour in ultraviolet —NH ₃ +NH ₃	<i>R_f</i> values in:				<i>λ</i> _{max} in ethanol	Compound
		30 per cent acetic	'Fores' tal'	Phenol PhOH			
I	Blue	0.13	0.90	—	~ 265, 272, 279, 320	~ 265, 272, 279, 320	Isoflavone*
II	Brown	0.49	0.74	0.85	267, ~ 295, 347	267, ~ 295, 347	3-O-Methyl-kaempferol
III	Brown—yellow	0.43	0.67	0.27	257, ~ 287, 295, ~ 355	257, ~ 287, 295, ~ 355	Quercetin 3,4'-diglucoside
IV	— Green	0.66	0.70	—	279, ~ 279, 330	279, ~ 279, 330	Flavone glycoside*

~ Indicates a shoulder. * Unidentified compounds.

Table 2. FLAVONOIDS AND CAROTENOIDS OF *P. icarus* AND THOSE OF *O. repens*

	Imagines		<i>O. repens</i>
	English	French	
Carotenoids			
Phytoene	+	+	+
α-Carotene	+	+	+
β-Carotene	—	—	+
γ-Carotene	+	—	+
Lutein	+	+	+
Lutein epoxide	—	+	—
Neoxanthin	—	+	+
Auroxanthin	—	—	+
Flavoxanthin	+	+	+
Flavonoids			
Isoflavone	—	+	+
3-O-Methyl-kaempferol	—	+	—
Quercetin 3,4'-diglucoside	—	+	—
Flavone glycoside	—	+	—

Carotenoids were extracted in methanol and partitioned with diethyl ether. The ethereal extracts were reduced *in vacuo* at about 40°, taken up in a small volume of *n*-hexane, separated by column chromatography and identified by our usual methods⁶. The results for carotenoids and flavonoids in the French and English butterflies of *P. icarus* and those of *O. repens* are given in Table 2. We could not obtain the food plant of the French species.

Phytoene was found in insects of both countries as well as in the food plant *O. repens*. As far as we know, this is the first record of this colourless C₄₀ polyene in insects even though it is found quite abundantly in a great number of plants. Contrary to what was found in certain other insects, such as *Schistocerca gregaria* and *Locusta migratoria migratoroides* R. and F.⁷, the carotenoids of the food plants of *P. icarus* are unchanged in the imagines even though not all the carotenoids present in *O. repens* were identified in the imagines. As for the flavonoids, the unknown isoflavone was obtained in the French butterflies and in *O. repens* but not in the English ones. Flavonoids were not identified at all in the English butterflies, but small amounts were present because only 100 specimens were used. Isoflavones are found mainly in the Leguminosae, and the presence of one such compound in the butterflies shows that the compounds are obtained by the insects from the particular food plant. The flavonols quercetin and kaempferol were identified in the French butterflies, though not found in *O. repens*. These compounds are well known in closely related members of the Leguminosae⁸ and it is quite likely that they are also found in *Anthyllis*. The larvae of *M. galathea*, however, are known to feed on certain grasses that contain tricetin and orientin but lack quercetin. Harborne⁵ has explained the absence of orientin in *C. pamphilus* in suggesting that, because orientin contains a catechol nucleus, it may be more readily metabolized by insects. This does not, however, explain its presence in *M. galathea* and, further, in our investigation we identified quercetin, which contains a catechol nucleus. Orientin might have been present in small amounts and not detected in *C. pamphilus*, as was the case with the English imagines in our investigation in which no flavonoids were identified, because the sample was smaller.

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