Phytochemistry, Vol. 25, No. 6, pp. 1309–1313, 1986.
Printed in Great Britain.

0031-9422/86 $3.00 + 0.00

FLAVONOID PIGMENTS IN SWALLOWTAIL BUTTERFLIES

ANGELA WILSON*

Department of Botany, Plant Science Labs, University of Reading, Whiteknights, Reading, Berks RG6 2AS, U.K.

(*Revised received* 23 *November* 1985)

Key Word Index—*Eurytides marcellus*; Papilionidae; swallowtail butterflies; *Asimina triloba*; Annonaceae; sequestration; flavonol glycosides.

Abstract—Flavonoid pigments have been identified in the swallowtail butterfly *Eurytides marcellus* and its larval foodplant *Asimina triloba* (Annonaceae). Although quercetin 3-glycoside, quercetin 3-rutinoside and quercetin 3-rutinoside-7-glucoside are present in the plant, only quercetin 3-glucoside is sequestered by the insect. Flavonoids have also been found in 10 out of 27 other papilionid species examined. These were mainly 3- and 7-glycosides of the flavonols quercetin and kaempferol. The sequestration of flavonoids by papilionid butterflies appears to be related both to the phylogeny of the Papilionidae and to the choice of larval foodplants by the various phylogenetic groups.

INTRODUCTION

The sequestration of plant secondary compounds such as the cardenolides [1] and carotenoids [2] by insects has been well documented. In comparison relatively little is known concerning the sequestration of flavonoid pigments. Flavonoids have been recognised in insects on a number of occasions [3–10], but, in most cases identification of these pigments has either not been attempted or is subject to doubt.

Recently flavonoid pigments in the marbled white butterfly have been studied in some detail [8–10]. Eighteen flavonoids were identified in *Melanargia galathea* L. and the same flavonoid pattern found in a number of other *Melanargia* species [8]. In addition, the dietary origin of insect flavonoids has been confirmed, and it has been shown that flavonoids are not merely sequestered from the diet but are also partly metabolized [9].

In this paper the relationship between flavonoid pigments in the zebra swallowtail *Eurytides marcellus* Cr. [syn: *Graphium marcellus* (Cr.)] and its larval foodplant *Asimina triloba* (L.) Dun. has been examined. Furthermore flavonoids have been reported in a number of other papilionids.

RESULTS AND DISCUSSION

Although three flavonol glycosides, namely, quercetin 3-glucoside, quercetin 3-rutinoside and quercetin 3-rutinoside-7-glucoside, were identified in *A. triloba*, only quercetin 3-glucoside was sequestered by *E. marcellus*. The chromatographic and spectral properties of these compounds are given in Table 1. Why it is that only quercetin 3-glucoside is sequestered by *E. marcellus* is not known. One possible explanation is that *E. marcellus* or its gut flora may metabolize all ingested flavonoids to form quercetin 3-glucoside, which may or may not be required for a specific function in the insect. Alternatively, *E. marcellus* may selectively sequester quercetin 3-glucoside from the diet; all other flavonoids may be excreted or metabolized and then excreted. Clearly, further investigations involving feeding experiments are required to determine the fate of ingested flavonoids in this insect.

From the two-dimensional chromatograms and aglycone results (Tables 2 and 3) it appears that all the other flavonoid-containing papilionid butterflies examined contain derivatives of either quercetin or kaempferol, or of both. The position and colour [11] of the flavonoid components on the 2D-chromatograms indicates that they are mainly 3-glycosides, 7-glycosides or the free aglycones.

Ford [3, 4] reported flavonoids in 141 out of the 365 papilionid species (38.6%; 5 of 16 genera), examined. Of these 96 were from the genus *Graphium*, 29 from *Parnassius*, two from *Lamproptera*, one from *Euryades* and 13 from the New World *Atrophaneura*. With the transfer of some of these *Graphium* species to other genera Ford's data has been revised and presented with the present data in Table 4.

In this study flavonoids have been found in 11 of the 28 papilionid species (39.3%) examined (Table 4). Four of these, namely *G. sarpedon*, *G. taboranus*, *G. angolanus* and *P. machaon*, have been reported to contain flavonoids for the first time. Combining these results with those of Ford [3, 4] means that a total of 372 papilionid species have now been examined of which 145 (38.9%), representing eight out of 21 genera, contain flavonoids (Table 4).

Although more recent investigations have largely superseded Ford's classification of the Papilionidae [12, 13], the results of this investigation support Ford's conclusion that flavonoid pigments are associated more with certain papilionid genera than others. At present it appears that *Papilio* (with the exception of *P. machaon*), *Troides*, Old World species of *Atrophaneura*, *Iphiclides*, *Baronia*, *Cressida*, *Teinopalpus*, *Luehdorfia*, *Bhutanitis*, *Sericinus*, *Zerynthia*, *Hypermnestra*, *Archon*, *Pachliopta* and *Battus* lack flavonoids, whereas *Eurytides*, *Protographium*, *Graphium*, *Euryades*, *Lamproptera*, *Parnassius* and species

*Present Address: Department of Zoology, University of Oxford, South Parks Road, Oxford OX1 3PS.

Table 1. R_f and spectral data, and hydrolysis products of flavonoids from *Eurytides marcellus* and *Asimina triloba*

Flavonoid	R_f (×100) in					λ_{max}^{MeOH}	Δ λ (μm)			Acid hydrolysis		Flavonoid
	BAW	15%	H_2O	PhOH	COL		+NaOH	+NaOAc	$+H_3BO_3$	Aglycone	Sugar	
Eurytides marcellus												
1	58	40	10	52	D/Y	258, 363	54	11	15	Quercetin	Glucose	Quercetin 3-glucoside
Asimina triloba.												
A	59	41	09	53	D/Y	259, 363	52	11	17	Quercetin	Glucose	Quercetin 3-glucoside
B	43	55	25	47	D/Y	259, 365	57	11	20	Quercetin	Rhamnose Glucose	Quercetin 3-rutinoside
C	30	70	41	23	D/Y	258, 361	51	0	20	Quercetin	Rhamnose Glucose	Quercetin 3-rutinoside-7-glucoside

Key: COL = colour in UV light + NH_3; D = dark absorbing; Y = yellow.

Table 2. R_f data for flavonoid components on 2D-chromatograms of *Asimina triloba* and the papilionid butterflies

Flavonoid component	R_f (×100) in		COL
	BAW	15% HOAc	
Asimina triloba			
A	57	46	D/Y
B	49	65	D/Y
C	26	77	D/Y
R	44	59	D/Y
Eurytides marcellus			
1	55	46	D/Y
R	44	55	D/Y
E. lysithous f. *rurik*			
1	57	43	D/Y
2	68	50	D/Y
R	46	54	D/Y
Graphium sarpedon			
1	54	44	D/Y
R	56	43	D/Y
G. agamemnon			
1	57	40	D/Y
R	55	46	D/Y
G. porthaon			
1	72	04	Y/Y
2	37	08	Y/Y
3	58	48	D/Y
R	45	56	D/Y
G. polistratus			
1	50	46	D/Y
R	58	44	D/Y
G. leonidas			
1	71	03	Y/Y
2	42	09	Y/Y
3	58	47	D/Y
R	42	55	D/Y
G. almansor			
1	77	54	D/Y
R	47	55	D/Y
G. taboranus			
1	74	05	Y/Y
2	48	10	Y/Y
3	52	41	D/Y
4	64	53	D/Y
R	46	54	D/Y
G. angolanus			
1	76	06	Y/Y
2	50	13	Y/Y
3	56	49	D/Y
4	69	56	D/Y
R	45	55	D/Y
Papilio machaon			
1	46	61	D/Y
R	45	56	D/Y

Key: A = quercetin 3-glucoside; B = quercetin 3-rutinoside; C = quercetin 3-rutinoside-7-glucoside; R = rutin (quercetin 3-rutinoside) marker; COL = colour in UV light + NH_3; D = dark absorbing; Y = yellow.

Table 3. The flavonoid aglycones, larval food plant family, and source of papilionid butterflies.

Species	Flavonoid aglyone	Larval foodplant family	Source (Site and collector's name)
Eurytides marcellus Cr.	Qu	Annonaceae	Virginia, U.S.A. (DW)
E. lysithous f. *rurik* Hbn.	Qu	Annonaceae	Santa Catarina, Brazil (DW)
Graphium sarpedon L.	Qu*	Lauraceae	Sri Lanka (TD)
G. agamemnon L.	Qu	Annonaceae	India (TD)
G. porthaon Hew.	Qu	Annonaceae	Livingstone, Zambia (TD)
G. polistratus G-Smith	Qu	Annonaceae	Malawi (TD)
G. leonidas Fab.	Qu	Annonaceae	Zambia (TD)
G. almansor Hon.	Km	Annonaceae	Zambia (TD)
G. taboranus Oberth.	Qu, Km*	Annonaceae	Zambia (TD)
G. angolanus Goeze.	Qu, Km*	Annonaceae	Zambia (TD)
Papilio agestor Gray	—	Lauraceae	Zambia (TD)
P. clytia L.	—	Lauraceae	Sri Lanka (TD)
P. polytes L.	—	Rutaceae	Sri Lanka (TD)
P. polymnestor Cr.	—	Rutaceae	Sri Lanka (TD)
P. ophidicephulus Oberth.	—	Rutaceae	Malawi (TD)
P. demodocus Esp.	—	Umbelliferae Rutaceae	Zambia (TD)
P. machaon L.	Qu*	Umbelliferae	Norfolk, England (AW)
P. polyxenes Fab.	—	Umbelliferae	Virginia, U.S.A. (DW)
P. phorcas Cr.	—	—	Zambia (TD)
P. constantinus Ward	—	Rutaceae	Zambia (TD)
P. zoroastres Druce	—	—	Zambia (TD)
P. fuelleborni Karsch	—	—	Malawi (TD)
P. nireus L.	—	Rutaceae	Zambia (TD)
P. glaucus L.	—	Magnoliaceae Rosaceae	Virginia, U.S.A. (DW)
Pachlioptera aristolochiae Fab.	—	Aristolochiaceae	Sri Lanka (TD)
Pachlioptera hector L.	—	Aristolochiaceae	India (TD)
Troides darsius Gray	—	Aristolochiaceae	Sri Lanka (TD)
Battus philenor L.	—	Aristolochiaceae	Virginia, U.S.A. (DW)

Key: Qu = quercetin; Km = kaempferol; — = flavonoids not detected; TD = Mr. Tim Dening; DW = Dr. David West; AW = the author. * = species reported to contain flavonoids for the first time.

of the New World *Atrophaneura* contain flavonoids (Table 4).

Generally papilionid butterflies can be grouped according to their larval foodplants, as in Table 5. From this, it would seem that the sequestration of flavonoids by papilionid butterflies is related both to the phylogeny of the Papilionidae and to the choice of larval foodplants by the various phylogenetic groups. Despite the presence of flavonoids in all the larval foodplant families it is not known why papilionid butterflies sequester flavonoids from some plant families but not from others.

It is generally believed that butterflies and the flowering plants originated at the same time, and that the swallowtails represent the first family of true butterflies [14, 15]. As such it is hardly surprising that some of the more primitive plant families, including the Magnoliaceae, Lauraceae, Annonaceae and Aristolochiaceae, seem to provide the majority of the larval foodplant species for the Papilionidae. The utilization of two other families, the Rutaceae and the Umbelliferae, which are not primitive can be related to (i) their distribution, which in the former is both tropical and temperate, whilst in the latter is temperate extending into cold regions, thus enabling the Papilionidae to extend its distribution and to colonize non-tropical areas, and (ii) their chemical similarity to other papilionid host plants. For example, the Rutaceae, Umbelliferae and certain species of the Lauraceae, have in common the presence of essential oils [16], whilst alkaloids such as berberine, magnoflorine and menispermine, are a feature of the Aristolochiaceae, Magnoliaceae, Annonaceae, Lauraceae and *Zanthoxylum* species of the Rutaceae. The presence of flavonols in these plant families presents a further similarity in their secondary chemistry.

A relatively recent illustration of the ability of papilionid butterflies to switch from one plant family to another is provided by the exploitation of the citrus crop (Rutaceae) in California as a larval food plant by the anise swallowtail *Papilio zelicaon* Lucas, which normally feeds on sweet fennel *Foeniculum vulgare* Mill. (Umbelliferae) [16]. The extent of this switch was so great that *P. zelicaon* become a serious pest in the orange orchards, and control measures were necessary. One of the key factors permitting the utilization of these two families as larval food plants appears to be the common possession of certain essential oils which act as larval feeding stimulants.

It has been suggested that flavonoids may have a role in wing pigmentation [10]. Support for such a role in the Papilionidae is provided by the presence of flavonoids in the yellow-marked forms of *Graphium* and *Atrophaneura* and their absence from the white-marked forms [1, 2]. Interestingly Ford [1] also reported the presence of a pale yellow fluorescent pigment in species lacking flavonoids.

Table 4. The occurrence of flavonoids in the Papilionidae from the results of Ford [3, 4] and the author combined

Papilionidae Genera	Ford's results				Author's results				No. of new species examined	Both results combined			
	No. of species				No. of species					No. of species			
	+	−	Total	%+	+	−	Total	%+		+	−	Total	%+
Papilio	0	117	117	0	1	13	14	7.1	1	1	117*	118*	0.85
Troides	0	21	21	0	0	1	1	0	0	0	21*	21*	0
Atrophaneura†	13	57	70	18.6	—	—	—	—	—	13	57	70	18.6
Eurytides	33	9	42	78.6	2	0	2	100	0	33	9	42	78.6
Protographium	1	0	1	100	—	—	—	—	—	1	0	1	100
Iphiclides	0	2	2	0	—	—	—	—	—	0	2	2	0
Graphium	62	4	66	93.9	8	0	8	100	3	65	4	69	94.2
Euryades	1	1	2	50	—	—	—	—	—	1	1	2	50
Baronia	0	1	1	0	—	—	—	—	—	0	1	1	0
Cressida	0	1	1	0	—	—	—	—	—	0	1	1	0
Lamproptera	2	0	2	100	—	—	—	—	—	2	0	2	100
Teinopalpus	0	1	1	0	—	—	—	—	—	0	1	1	0
Luehdorfia	0	2	2	0	—	—	—	—	—	0	2	2	0
Bhutanitis	0	2	2	0	—	—	—	—	—	0	2	2	0
Sericinus	0	1	1	0	—	—	—	—	—	0	1	1	0
Zerynthia	0	3	3	0	—	—	—	—	—	0	3	3	0
Hypermnestra	0	1	1	0	—	—	—	—	—	0	1	1	0
Archon‡	0	1	1	0	—	—	—	—	—	0	1	1	0
Parnassius	29	0	29	100	—	—	—	—	—	29	0	29	100
Pachliopta	—	—	—	—	0	2	2	0	2	0	2	2	0
Battus	—	—	—	—	0	1	1	0	1	0	1	1	0
Total	141	224	365	38.6	11	17	28	39.3	7	145	227	372	38.9

*As Ford [3, 4] did not record the names of papilionids lacking flavonoids, it is not certain if the species examined in this study were examined before.
†*Atrophaneura*, syn: *Polydorus*.
‡*Archon*, syn: *Doritis*.

Table 5. The presence of flavonoids in swallowtail groups* and their larval food plant families

Swallowtail group	Larval foodplant family	Presence of flavonoids in butterflies
Papilio (Old World swallowtail group) e.g. *P. polyxenes* Fab.	Umbelliferae, with offshoots to the Compositae and Rutaceae.	— (except for *P. machaon*)
Papilio (tiger swallowtails and allies) e.g. *P. glaucus* L.	Magnoliaceae, Salicaceae, Oleaceae, Rosaceae, Lauraceae, Rutaceae, Rhamnaceae and others	–
Papilio (thoas and anchisiades group) e.g. *P. cresphontes* Cr. and *P. ornythion* Bsdv.	Rutaceae	—
Eurytides (kite swallowtails) e.g. *E. marcellus* Cr.	Annonaceae, Lauraceae and Magnoliaceae	+
Parides and *Battus* cattle hearts and gold rims) e.g. *B. philenor* L.	Aristolochiaceae, Rutaceae and Piperaceae	–
Subfamily Parnassiinae (parnassians) e.g. *Parnassius* species.	Aristolochiaceae, Saxifragaceae, Crassulaceae and Fumariaceae	+
Subfamily Baroniinae (short-horned swallowtail). Single species *Baronia brevicornis* Salv.	Leguminosae (*Acacia cymbispina* Sprague & Riley)	–

*Swallowtail groups are from ref. [14].

Hence it is possible that the sequestration of flavonoids by various papilionids is related to the way(s) in which wing colour is produced; some pigments are synthesized *de novo* by the insect themselves, whereas others are sequestered from the larval foodplants.

EXPERIMENTAL

Eurytides marcellus Cr. reared on *A. triloba* (L.) Dun. in the laboratory at Virginia State Polytechnic and University, U.S.A. were killed, placed in small paper envelopes and air-mailed to the author by Dr. D. West. Air-dried *A. triloba* collected from Montgommery County, VA, U.S.A. was dispatched as above.

Extracts and chromatography. Flavonoids were extracted from 25 *E. marcellus* by soaking the crushed tissues in *ca* 50 ml 70% EtOH at room temp. for 24 hr. After removal of this extract the tissues were extracted × 5 with warm 70% EtOH, the extracts combined, filtered using Whatman No. 1 filter paper, washed with petrol (bp 40–60°) and concd to a small vol. under red. pres. In the same way flavonoids were extracted from *A. triloba*. Flavonoids were extracted from butterflies listed in Table 3 by soaking the crushed tissues in 1–2 ml 70% EtOH in small sample tubes at room temp. for 24 hr. A small aliquot of each extract was used to prepare a 2D-chromatogram run in BAW (*n*-BuOH–HOAc–H_2O, 4:1:5, upper phase) and 15% aq. HOAc. A further aliquot of each extract was hydrolysed with 2 N HCl at 100° for 30 min, the cooled hydrolysate extracted twice with EtOAc, the extracts combined, evaporated to dryness, and the residue dissolved in a few drops of 90% EtOH. Aglycones were identified by their chromatographic properties in BAW (4:1:5), FOR (HOAc–conc. HCl–H_2O, 30:3:1), CAW ($CHCl_3$–HOAc–H_2O, 30:15:2), and PhOH (phenol–H_2O, 4:1); their colours in UV light; and by co-chromatography with authentic samples.

Standard procedures were used for the separation, purification and identification of flavonoids [11, 17, 18]. Flavonoids from *E. marcellus* and *A. triloba* were separated and purified by prep. PC. Known pigments were identified on the basis of R_f; UV spectral analysis; acid and enzymic hydrolyses to aglycone and sugars; and by direct comparison with authentic samples where possible.

Acknowledgements—This work was performed as part of a Ph.D. study at Reading University. I should like to thank the University of Reading for financial support, my supervisor Professor J. B. Harborne for his help and Dr. D. West (Virginia Polytechnic and State University, U.S.A) and Mr. T. Dening for kindly donating plant and butterfly material.

REFERENCES

1. Brower, L. P., Seiber, J. N., Nelson, C. J., Lynch, S. P., Hoggard, M. P. and Cohen, J. A. (1984) *J. Chem. Ecol.* **10**, 1823.
2. Kayser, H. (1982) in *Carotenoid Chemistry and Biochemistry*. (Britton, G. and Goodwin, T. W., eds) pp. 195–210. Pergamon Press, Oxford.
3. Ford, E. B. (1941) *Proc. R. Entomol. Soc. Lond.* (*A*) **16**, 65.
4. Ford, E. B. (1944) *Trans. R. Entomol. Soc. Lond.* **94**, 201.
5. Morris, S. J. and Thomson, R. H. (1963) *J. Insect Physiol.* **9**, 391.
6. Morris, S. J. and Thomson, R. H. (1964) *J. Insect Physiol.* **10**, 377.
7. Feltwell, J. S. and Valadon, L. R. G. (1970) *Nature* **225**, 969.
8. Wilson, A. (1985) *Phytochemistry* **24**, 1685.
9. Wilson, A. (1985) *J. Chem. Ecol.* **11**, 1161.
10. Wilson, A. (1986) *J. Chem. Ecol.* **12**, 49.
11. Harborne, J. B. (1973) *Phytochemical Methods*. Chapman & Hall, London.
12. Munroe, E. (1961) *Can. Entomol.* (*Suppl.*) **17**, 1.
13. Hancock, D. L. (1983) *Smithersia* **2**, 1.
14. Tyler, H. A. (1975) *Swallowtail Butterflies of North America*. Naturegraph, New York.
15. Kristensen, N. P. (1976) *Z. Zool. Syst. Evolution Forsch.* **14**, 25.
16. Shapiro, A. and Masuda, K. (1981) *New Scientist* **90**, 160.
17. Markham, K. R. (1980) *Techniques in Flavonoid Identification*. Academic Press, London.
18. Harborne, J. B. (1967) *Comparative Biochemistry of the Flavonoids*. Academic Press, London.