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FLAVONOID PIGMENTS OF BUTTERFLIES IN THE GENUS *MELANARGIA*

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Key Word Index—*Melanargia galathea*; Satyridae; marbled white butterfly; sequestration; flavonoids; tricin; vitexin; isovitexin; orientin; isoorientin; glucosides.

Abstract—Flavonoid pigments (18) were identified in the wings and body of *Melanargia galathea*: tricin, tricin 7-glucoside, tricin 7-diglucoside, tricin 4'-glucoside, luteolin, luteolin 7-glucoside, luteolin 7-diglucoside, luteolin 7-triglucoside, apigenin, apigenin 7-glucoside, orientin, orientin 7-glucoside, iso-orientin, iso-orientin 7-glucoside, vitexin 7-glucoside, isovitexin, isovitexin 7-glucoside and a novel but incompletely identified tricin 4'-conjugate. Examination of the wings and bodies of individual *M. galathea*, *M. galathea* var. *procida*, *M. lachesis*, *M. russiae*, *M. larissa*, *M. occitanica* and *M. ines* butterflies from a number of different populations in Europe by 2D PC revealed that variation in their flavonoid patterns was so minor that the flavonoid pattern of these *Melanargia* spp. may be considered constant. The concentration of flavonoids in the wings of each butterfly was greater than that in the body, as is the covering of scales. Not all flavonoids are located in the scales; some are also located in the reproductive tissues of the female. With the exception of the tricin 4'-conjugate which was absent from the egg and first instar larvae before feeding commences, these flavonoids were present in all the life stages of *M. galathea*. The presence of tricin 4'-conjugate in *Melanargia* but its absence from the larval food plants suggests that this compound is synthesized by the insect and that flavonoids are not merely sequestered from the diet but are also partly metabolized.

INTRODUCTION

Despite the well documented occurrence of flavonoid pigments in plants [1], little is known of their existence in animals. Flavonoids have been recognized in insects on a number of occasions but, in the main, identification of these pigments has either not been attempted or is subject to doubt. Compounds, reputedly flavonoid, have been reported in the cocoons of the silkworms *Bombyx mori* L. [2, 3] and *Theophila mandarina* Moore [4], though none of these substances has been fully identified.

That flavonoids are by no means rare in the Lepidoptera, but are associated more with certain families than others, was demonstrated by Ford [5, 6]. Although his results were confined to simple colour tests, not free from ambiguity, flavonoids were found in 36 of 327 butterfly genera (11.5%, six of 11 families) and in 10 of 192 moth genera (5%, four of 13 families) examined. From the wings of *M. galathea* L. Thomson [7] isolated a small amount of material which he considered to be quercetin. A similar pigment was isolated from the grass *Dactylis glomerata* L., a possible larval food plant of this butterfly [8].

Morris and Thomson [9] provided the first positive identification of flavonoids in the animal kingdom. From the wing extracts of 400 *M. galathea*, the major flavones present were identified as tricin, tricin glycosides and an orientin glycoside. The absence of any flavonols indicated that Thomson's [7] report of quercetin in this insect was incorrect. In a parallel study on the flavonoids of the small heath butterfly *Coenonympha pamphilus* L., tricin, tricin glycosides and traces of other flavonoids were identified [10]. The occurrence of tricin and orientin in both *M. galathea* and in many of its grass larval food plant species suggested to Morris and Thomson that the insect flavonoids were most likely of a dietary origin.

Feltwell and Valadon [11] tentatively identified the free flavonol 3-*O*-methylkaempferol and the flavonol glycoside quercetin 3,4'-diglucoside in the common blue butterfly, *Polyommatus icarus* Rott. An unidentified flavone glycoside and an unidentified isoflavone were also obtained.

Flavonoids in the wings of individual butterflies can be recognized by either fuming the specimen with ammonia vapour, or extracting them with ethyl acetate and shaking the extract with alkali. A yellow colouration in both instances indicates their presence [5]. Most of the work on insect flavonoids though has involved the extraction of large numbers, e.g. 400 *M. galathea* [9], 600 *C. pamphilus* [10] and 800 *P. icarus* [11]. Consequently, little is known concerning the flavonoid pigments of individual butterflies; for instance, do conspecifics contain the same flavonoids?

In this paper some further flavonoid pigments have been identified in *M. galathea* and the occurrence of flavonoids in the egg, larval and pupal stages reported for the first time. Furthermore, the same flavonoid pattern has been found to occur in *M. galathea* var. *procida*, *M. lachesis*, *M. russiae*, *M. larissa*, *M. occitanica* and *M. ines*.

RESULTS

A total of 18 flavonoids were identified in the wings and body of *M. galathea*, with the concentration of pigments greatest in the former. The chromatographic and spectral properties and identities of these flavonoids can be seen in Table 1. The flavonoids present in each of the 2D chromatogram spots are given in Table 2.

Morris and Thomson's report [9] of tricin, tricin

Table 1. R_f and spectral data for the flavonoids of *Melanargia galathea*

Compound No.	R_f (×100) in					UV λ_{max}^{MeOH} (nm)	UV $\Delta\lambda$ (μm)				Flavonoid
	BAW	15% HOAc	H_2O	PhOH	BEW		+NaOH	+NaOAc	$+H_3BO_3$	$+AlCl_3$	
1.0	78	04	00	96	68	249, 270, 354	68	6	0	24	Tricin
1.1	79	05	00	67	86	258, 270, 352	51	12	25	41	Luteolin
1.2	86	09	00	93	82	270, 339	61	5	0	43	Apigenin
2.0	27	13	18	53	16	270, 348	54	0	0	26	Tricin 7-glucoside
2.1	27	17	06	38	—	272, 347	53	6	14	41	Orientin
3.0	43	35	07	52	—	272, 352	58	0	30	42	Luteolin 7-diglucoside
3.1	47	42	10	59	43	272, 355	55	0	25	41	Orientin 7-glucoside
3.2	52	35	11	90	62	274, 330	50	0	0	43	Vitexin 7-glucoside
3.3	44	37	10	55	24	271, 345	63	5	30	44	Iso-orientin
3.4	53	41	17	80	—	272, 331	54	4	0	46	Isovitexin
4.0	41	54	46	61	48	273, 326	42	9	0	27	Tricin 4′-conjugate
5.0	40	16	02	57	25	270, 352	56	0	26	42	Luteolin 7-glucoside
5.1	51	23	16	75	63	273, 330	70	0	0	47	Apigenin 7-glucoside
6.0	23	20	29	48	15	271, 350	53	0	0	29	Tricin 7-diglucoside
7.0	24	52	16	48	27	273, 352	52	0	26	44	Luteolin 7-triglucoside
7.1	26	45	10	45	25	272, 351	55	0	25	44	Iso-orientin 7-glucoside
8.0	38	68	32	62	35	273, 332	56	0	0	44	Isovitexin 7-glucoside
9.0	86	27	03	95	90	273, 330	14	5	0	24	Tricin 4′-glucoside

Table 2. The flavonoid components of spots on the 2D chromatograms of *Melanargia galathea*

Spot No.	R_f (× 100) in BAW	15% HOAc	COL in UV ± NH_3	Flavonoid components of spots
1	71	07	D/Y	Tricin, luteolin, apigenin
2	35	10	D/Y	Tricin 7-glucoside, orientin
3	46	41	D/Y	Luteolin 7-diglucoside, orientin 7-glucoside, vitexin 7-glucoside, iso-orientin, isovitexin
4	41	54	D/D	Tricin 4'-conjugate.
5	49	16	D/Y	Luteolin 7-glucoside, apigenin 7-glucoside
6	23	20	D/Y	Tricin 7-diglucoside
7	31	45	D/Y	Luteolin 7-triglucoside, iso-orientin 7-glucoside.
8	37	68	D/Y	Isovitexin 7-glucoside
9	80	22	D/Y	Tricin 4'-glucoside
R	41	57	D/Y	Rutin marker

R, Rutin; Y, yellow; D, dark.

glycosides and an orientin glycoside in the wings of *M. galathea* has been confirmed and the formerly incompletely identified glycosides are now shown to be tricin 7-glucoside, tricin 7-diglucoside and orientin 7-glucoside. The remaining 14 compounds namely, luteolin, luteolin 7-glucoside, luteolin 7-diglucoside, luteolin 7-triglucoside, apigenin, apigenin 7-glucoside, orientin, iso-orientin, iso-orientin 7-glucoside, isovitexin, isovitexin 7-glucoside, vitexin 7-glucoside, tricin 4'-glucoside and an incompletely identified tricin 4'-conjugate, have been identified from an insect for the first time. No free vitexin was found even though a vitexin glycoside was present. Luteolin 7-triglucoside and the tricin 4'-conjugate are novel compounds reported for the first time. Where the flavonoids identified contain more than one glucose residue it should be noted that, although the numbers of residues are known, the nature of the glucosidic links have still to be determined.

The spectral and colour properties of compound **4.0** (Table 2) indicated a substitution at the OH-4' position, but acid hydrolysis yielded only tricin. An identical electrophoretic mobility to that of quercetin 3-sulphate, suggested a negatively charged monosubstituted conjugate. Examination of the aqueous fraction of the hydrolysate for K^+ and HSO_4^- ions did not reveal either. As yet, this compound remains incompletely identified, but the data so far suggests a tricin 4'-conjugate possibly involving either sulphate or phosphate. Tricin 4'-conjugate was not found in any of 30 possible larval food plant grass species examined electrophoretically.

The most abundant flavonoids in *M. galathea* were tricin, tricin 7-glucoside, orientin 7-glucoside, vitexin 7-glucoside and the tricin 4'-conjugate. The remaining flavonoids were present in approximately equal amounts. All the spot components occupying the same positions on the *Melanargia* 2D chromatograms were found to be the same, permitting the direct comparison of chromatograms. The 2D chromatographic flavonoid patterns of all the life stages of *M. galathea* were identical except for the absence of the tricin 4'-*O*-conjugate from the whole eggs, eggshells, first instar pharate larvae and the first instar larvae before feeding on the larval food plants had begun (Table 3). This result was confirmed by the absence of any electrophoretically mobile flavonoids in these life stages.

The presence or absence of each flavonoid spot on the 2D chromatograms of the wings and bodies of every butterfly examined was recorded. In Table 4 these data have been summarized to give the numbers of male and female butterflies from each population with each of flavonoid spots 1–9 present on their 2D chromatograms. Only very minor variation was found in the flavonoid content of *M. galathea* from each population examined. The flavonoids in spots 1–9, were present in the wings of every butterfly examined whereas, in the bodies, although the flavonoids in spots 1–5 were always present, those in spots 6–9 were occasionally absent.

Table 3. The occurrence and electrophoretic mobility of flavonoids in the life stages of *Melanargia galathea*

M. galathea Life stage	Presence or absence of flavonoid spot 1	2	3	4	5	6	7	8	9	Electrophoretic mobility
First instar pharate larvae	+	+	+	−	+	+	+	+	+	None
Eggshell	+	+	+	−	+	+	+	+	+	None
Whole egg	+	+	+	−	+	+	+	+	+	None
First instar larvae before feeding on grasses	+	+	+	−	+	+	+	+	+	None
First instar larvae feeding on grasses	+	+	+	+	+	+	+	+	+	1.0
Second instar larvae	+	+	+	+	+	+	+	+	+	1.0
Third instar larvae	+	+	+	+	+	+	+	+	+	1.0
Fully grown fourth instar larvae	+	+	+	+	+	+	+	+	+	1.0
Imago	+	+	+	+	+	+	+	+	+	1.0

Table 4. The occurrence of flavonoids in the wings and bodies of *Melanargia* butterflies

Population and species	Sex	No. of butterflies with each of flavonoid spots 1–9 present on their 2D chromatograms: Flavonoid spots on wings 1–9	Flavonoid spots on body 1–5	6	7	8	9	No. of butterflies examined
M galathea								
North Moreton,	Male	27	27	22	24	24	23	27
Oxon, U.K.	Female	23	23	22	25	22	20	23
Wayland Smithy,	Male	15	15	11	12	10	11	15
Berks, U.K.	Female	10	10	7	10	5	6	10
Barbury Castle,	Male	15	15	11	13	12	10	15
Wilts, U.K.	Female	10	10	9	8	6	7	10
Aston Tirrold,	Male	15	15	10	13	10	9	15
Oxon, U.K.	Female	10	10	8	8	7	6	10
Blewbury,	Male	3	3	2	2	1	2	3
Oxon, U.K.	Female	3	2	1	1	0	1	2
Salisbury Plain,	Male	2	3	1	1	0	1	2
Wilts, U.K.	Female	3	3	2	2	1	1	3
Petersfield,	Male	3	3	2	2	2	2	3
Hants, U.K.	Female	2	2	2	2	2	1	2
Sandown,	Male	3	3	1	3	3	1	3
Isle of Wight, U.K.	Female	2	2	1	1	1	1	2
Ragaz,	Male	3	3	2	2	0	2	3
Switzerland	Female	2	2	2	1	1	1	2
Karia,	Male	2	2	1	1	2	0	2
Greece	Female	3	3	2	2	2	2	3
Yugoslavia	Male	2	2	1	1	1	0	2
	Female	3	3	3	1	1	1	3
Heubach, West	Male	3	3	2	1	3	1	3
Germany	Female	2	2	0	2	0	1	2
Herbrechtingen,	Male	3	3	1	3	2	1	3
West Germany	Female	2	2	1	2	1	1	2
Neuffen,	Male	3	3	2	2	2	1	3
West Germany	Female	2	2	1	2	1	1	3
Marmagen,	Male	2	2	2	1	1	1	2
West Germany	Female	3	3	1	2	1	1	3
M. galathea var. *procida*								
Menaggio,	Male	6	6	6	4	4	5	6
Italy	Female	4	4	4	3	3	3	4
Promontogne,	Male	3	3	3	3	2	2	3
Switzerland	Female	2	2	2	1	2	1	2
M. lachesis								
Montpellier,	Male	3	3	2	2	1	2	3
France	Female	2	2	1	1	1	1	2
M. ines								
Amizmiz,	Male	2	2	1	1	0	1	2
Morocco	Female	3	3	3	3	2	1	3
M. larissa								
Erciyes Dagh,	Male	3	3	3	2	2	0	3
Turkey	Female	2	2	1	2	1	1	2
M. russiae								
Sicily	Male	3	3	2	2	2	2	3
	Female	2	2	2	1	1	1	2
M occitanica								
France	Male	3	3	2	3	0	1	3
	Female	2	2	2	2	2	0	2

As the concentration of flavonoids in the butterfly body is lower than that in the wings, some of the more minor flavonoids, particularly those in spots 6–9, were more difficult to detect. It is likely, therefore, that on the occasions when these spots have been recorded as absent from the body, they may actually have been present but in very low concentrations and, therefore, not detected.

The flavonoids in spots 1–9 were present in the wings of

all the *M. galathea* var. *procida*, *M. lachesis*, *M. russiae*, *M. larissa*, *M. occitanica* and *M. ines* butterflies examined. In the bodies of these butterflies the flavonoid in spots 1–5 were always present whilst those in spots 6–9 were sometimes absent. The flavonoid patterns of these species and varieties are identical to that of *M. galathea*. Variation in the flavonoid contents of the *Melanargia* spp. examined, representing 25% of the genus, is so minor that their flavonoid pattern may be considered constant.

DISCUSSION

The glycoflavones and flavone aglycones identified in *M. galathea* are characteristic of those commonly found in grasses [12]. This similarity between the flavonoids of the butterfly and its larval food plants, supports the idea that the insect flavonoids are of a dietary origin. The absence of flavonols in *M. galathea*, despite their presence in a number of grass species found in the typical *M. galathea* habitat, e.g. *Lolium perenne* L. and *Festuca pratensis* Huds. [12], is somewhat surprising.

The glycosylation of *M. galathea* flavonoids at only the OH-7 or OH-4′ positions, suggests that either specific glycosylation pathways operate within the insect, or that only flavonoids glucosylated at these positions are sequestered from the diet. Since the hydrolysis of many glycosides is likely during feeding and subsequent digestion, a combination of both pathways may operate. Dietary aglycones may undergo specific glucosylations, whilst those glycosides more resistant to hydrolysis, i.e. the 7- and 4′- glycosides compared to the 5-glycosides [13], may be sequestered unchanged from the diet. The reasons, however, for the limited sequestration of glucosides by this insect are not known.

In the life stages of *M. galathea* prior to feeding on the larval food plants, flavonoids cannot have been obtained directly from the diet, but must have been derived from the female butterfly and transmitted via the developmental materials to the egg. It, therefore, follows that not all of the flavonoids in *M. galathea* are located in the scales since some must also be located in the reproductive tissues, particularly those of the female.

The sequestration and location of secondary plant substances in the reproductive organs of *M. galathea* is not peculiar to this insect. Carotenoids are located in the reproductive organs and eggs of a range of insects, including members of the Lepidoptera [14, 15], whilst pyrrolizidine alkaloids occur in both the body and eggs of the tiger moth, *Arctia caja* L. [16, 17].

That the tricin 4′-conjugate is synthesized by the insect itself, probably by a combination of tricin from the larval diet with the conjugate ion obtained from the insect, is suggested by its absence from any of the likely larval food plants. Hence, flavonoids are not merely sequestered from the larval diet but are also partly metabolized. In addition to the free tricin present in a number of grass species [18], further quantities are likely to be generated during larval feeding. It has been shown that the free flavonoid aglycones are more toxic to insects than their corresponding glycosides [19]. As sulphate conjugation is recognized as a minor detoxification mechanism of foreign phenols in insect tissues [20], the conjugation of tricin with an as yet unidentified anion at the OH-4′ position may provide a means of inactivating potentially harmful tricin encountered in the larval diet.

That each *Melanargia* butterfly examined contained exactly the same flavonoids is somewhat surprising since the larvae of *Melanargia* are reported to be generalist grass feeders, feeding on a variety of grass species [21–23], each of which contains a different combination of flavonoids. However, the hypothesis that *Melanargia* larvae are generalist grass feeders has never been proven, based only on the assumption that one grass is much the same as another as food for the larvae. This though is not the case, as each grass species differs in the degree of pubescence on the leaf surface, the toughness of the cuticle, the presence of silica particles in the leaf tissues, the levels of available nutrients and in its secondary chemistry, all of which have been shown, in recent years, to be extremely important in an insect's choice of food [24]. In this laboratory a number of grass species have been used to rear *M. galathea* [25; P. S. Wagener, personal communication] but, generally, the survival rate is very low. The results of these experiments indicate that *M. galathea* is much more specialized, feeding only on *Festuca* spp. species and, hence, a constant *Melanargia* flavonoid pattern is, thus, produced in the wild.

Although Ford [5] found the distribution of flavonoids in the Lepidoptera useful in the systematics of the order as a whole, the presence of flavonoids in the genus *Melanargia* is of no value for distinguishing between the species so far examined as they all have exactly the same flavonoids. The genetically controlled variation in the extent of the black markings on the wings, which is characteristic of the different *Melanargia* varieties and species [22], appears to have no qualitative effect on the sequestration of flavonoids.

EXPERIMENTAL

Melanargia *material*. Adult *M. galathea* were collected during July and August 1981 from a site at North Moreton in Oxfordshire. Live butterflies were transported to the laboratory in dark boxes where they were maintained in 1 × 1 m cages constructed of wooden frames and white netting, and fed on diluted honey. Male *M. galathea* were usually killed 2–3 days after capture and the females 1–2 weeks after capture, using EtOAc vapour. Eggs, laid by the caged females, were collected daily and placed in Petri dishes so providing material for examination of the whole egg, eggshell, first instar pharate larvae (unhatched) and the first instar larvae immediately after hatching but before feeding.

The remaining life stages were collected by visiting the site at appropriate times of the year and searching among the grasses. As the larvae are night feeders the most productive collecting time was dusk. First instar larvae were collected in January, second instar larvae in February, third instar in March and the full grown fourth instar larvae in May. Larvae collected from the wild were starved for 24 hr before extraction to remove any plant material present in their gut which might influence the flavonoid results. Pupae were collected by searching amongst the bases of grass stems during June.

Larval and pupal identifications were verified by comparison of their characteristics with those recorded for *M. galathea* in the lit. [21, 23], by comparison with laboratory reared specimens and by rearing some of the collected material through to the adult stage.

Material for examination of the first instar pharate larvae (unhatched embryo) and eggshells was prepared by tearing a hole in the shell of 18-day-old eggs and removing the developing larvae using a small pin. The pharate larva at this stage was almost

fully developed and easily separated from the eggshell. Hatching normally occurs at 21 days.

British *M. galathea* were collected from sites at North Moreton, Aston Tirrold and Blewbury in Oxfordshire, U.K., Wayland Smithy in Berkshire, U.K., Barbury Castle and Salisbury Plain in Wiltshire, U.K., Sandown on the Isle of Wight, U.K. and Petersfield in Hampshire, U.K. *Melanargia galathea* from Karia, Mt Olympos, Greece, Heubach, in Baden-Wurttemberg on the Schwabische Alb, ESE. of Schwabisch-Grund, Herbrechtingen in Baden-Wurttemberg on the Schwabische Alb S. of Heidenheim, West Germany; Neuffen, in Baden-Wurttemberg on the Schwabische Alb SSE. of Stuttgart, West Germany; Marmagen, in Nordrhein-Westfalen, in the Eifel, SE. of Bonn, West Germany; *M. larissa* Geyer, from the Erciyes Dagh, Turkey; *M. occitanica* Esper, from the Alpes maritimes, S. France; and *M. russiae* Esper, from Bosco di Ficuzza, Sicily, were collected by Dr. P. S. Wagener; *M. galathea* from Yugoslavia by Mr. T. Dening; *M. lachesis* Hubner from Montpellier, France, by Dr. R. Tilley. *Malanargia galathea* from Ragaz and *M. galathea* var. *procida* Herbst. from Promontogne, in Switzerland; *M. galathea* var. *procida* from Menaggio, Italy; and *M. ines* Hoffmannsegg from Amizmiz, Morocco, were donated by Reading Museum.

Plant material. Fresh leaf material of the grass species listed in Table 4 was collected from the site at North Moreton, U.K. during April 1982. Voucher specimens of each species have been deposited in the herbarium of the Botany Department, University of Reading.

Extracts and chromatography. The body and wings of each butterfly were separated, placed in small sample tubes and the crushed tissues extracted with 1–2 ml 70% EtOH for 12 hr at room temp. Whole eggs (50) 75 empty eggshells, 75 first instar pharate larvae, 25 first instar larvae before feeding has begun, 10 first instar larvae after feeding has begun, five second instar larvae, four third instar larvae and three fourth instar larvae (each examined individually) were placed in small sample tubes and extracted as above.

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. The wing and body extracts of 400 *M. galathea* which produced similar chromatograms were combined to produce a body extract and a wing extract. These two extracts were treated separately throughout due to the relatively large quantities of substances in the body fraction which interfere with the chromatographic separation of flavonoids. Further extraction of the wing and body tissues was achieved by soaking them in *ca* 500 ml 70% EtOH at room temp. for 24 hr before removal of this extract followed by five successive extractions with warm 70% EtOH. The combined extracts were then filtered, washed with petrol (bp 40–60°) and concd to a small vol. under red. pres.

Flavonoid identification. Standard procedures were used foe the separation, purification and identification of the flavonoids [26, 27]. Prep. PC was used to separate and purify the flavonoids. Known pigments were identified on the basis of R_f, UV spectral analysis, and acid and enzymic hydrolyses to the aglycone and sugar, and by direct comparison with authentic samples where possible.

Identification of luteolin 7-triglucoside. Luteolin 7-triglucoside was identified by the production of a series of intermediate glucosides during acid hydrolysis, the products of which were sampled at regular time intervals. The chromatographic and spectral data of these intermediates are given in Table 5.

Comparison of spot components on the 2D chromatograms. Components occupying the same positions on the 2D chromatograms of the wings and bodies of male and female butterflies from each population and of the life stages of *M. galathea* were compared by eluting the spots from the chromatograms in 80% MeOH and examining them in five solvents: BAW (4:1:5); 15% HOAc; H_2O; PhOH; and BEW (*n*-BuOH–EtOH–H_2O, 4:1:2.2). Spot 1 on the chromatograms, which contained a mixture of flavone aglycones, was run in an extra solvent, FOR (HOAc–conc. HCl–H_2O, 30:3:1). Components which were the same gave identical mobilities in each of the solvent systems. The R_f data of the 2D chromatogram spot components in each of the solvent systems is given in Table 6.

Electrophoresis. An aliquot of each of the life stage extracts was examined for the presence of charged flavonoids by electrophoresis on Whatman No. 3 paper at pH 2.2 (HOAc–HCOOH buffer), for 2 hr, at 400 V/cm, with an authentic marker (quercetin 3-sulphate). Direct 70% EtOH extracts of each of the grass species were also examined.

Detection of K^+ and HSO_4^- ions in a flavonoid sulphate. Ions (K^+ and HSO_4^-), produced by hydrolysis of a flavonoid sulphate with 2 M HCl for 30 min at 100°, were detected qualitatively by TLC. Extraction of the hydrolysate with EtOAc removed the aglycones. After removal of the acid and concn, the aq. residue was dissolved in a few drops of H_2O and co-chromatographed with $KHSO_4$ on cellulose plates, developed in 20% 0.1 M HCl in EtOH. Spraying with sodium cobaltous hexanitrite, reveals K^+ as a yellow-green spot, R_f 0.16, and HSO_4^- as a white spot, on a pale yellow background.

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Table 5. R_f and spectral data of the hydrolysis products of luteolin 3-*O*-triglucoside

Hydrolysis time (min)	R_f (×100) in BAW	15%	H_2O	PhOH	UV λ_{max}^{MeOH} (nm)	UV Δλ (μm) +NaOH	+NaOAc	+H_3BO_3	COL in UV ±NH_3	Hydrolysis product
0	23	53	17	48	272, 352	53	0	25	D/Y	Luteolin 7-triglucoside
5	40	41	09	51	271, 352	57	0	29	D/Y	Luteolin 7-diglucoside
10	43	16	04	56	272, 351	57	0	27	D/Y	Luteolin 7-glucoside
40	79	02	00	67	256, 269, 352	56	12	26	D/Y	Luteolin

Y, yellow; D, dark.

Table 6. R_f data of the flavonoid components of spots on the *Malanargia galathea* 2D chromatograms after elution from the chromatograms

Spot No.	R_f (×100) in						No. of flavonoids in spot
	BAW	15% HOAc	H_2O	PhOH	BEW	FOR	
1	78, 83	05	00	68, 94	70, 84	65, 74, 87	3
2	25	10	05, 17	36, 51	20	—	2
3	45, 53	40	10, 18	53, 60 80, 90	11, 24, 40 54, 62	—	5
4	41	54	44	61	47	—	1
5	41, 52	15, 23	3, 15	56, 78	24, 61	—	2
6	23	20	30	49	16	—	1
7	25	49	10, 17	46	26	—	2
8	37	67	33	64	34	—	1
9	85	25	04	94	89	—	1
Rutin marker	42	49	21	48	45	—	1

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