the metapleural glands are inflated and secrete a sticky and repellent fluid [6, 7]. The defense mechanisms and venom chemistry could offer valuable characteristics to help in clarifying the taxonomy of this large genus. Work along these lines is in progress in our laboratories.

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1 Daloze, D., Braekman, J. C., Vanhecke, P., Boevé, J. L., and Pasteels, J. M., Can. J. Chem. *65* (1987) 432.
2 Pasteels, J. M., Daloze, D., and Boevé, J. L., J. chem. Ecol. *15* (1989) 1501.
3 Beroza, M., and Bierl, B. A., Analyt. Chem. *39* (1967) 1131.
4 Bierl-Leonhardt, B. A., De Vilbiss, E. D., and Plummer, J. R., J. Chromat. Sci. *18* (1980) 364.
5 Attygalle, A. B., and Morgan, E. D., Chem. Soc. Rev. *13* (1984) 245.
6 Blum, M. S., and Hermann, H. R., in: 'Arthropod Venoms', pp. 801–869. Ed. S. Bettini. Springer Verlag, N.Y., 1978.
7 Maschwitz, U., Oecologia *16* (1974) 303.
8 Attygalle, A. B., Siegel, B., Vostrowsky, O., Bestmann, H. J., and Maschwitz, U., J. chem. Ecol. *15* (1989) 317.

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Flavonoid wing pigments in grasshoppers

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Abstract. Yellow pigments extracted from the hindwings of *Dissoteira carolina* (L.) (Acrididae:Oedipodinae) were identified by HPLC, GLC, MS and absorbance spectra as primarily quercetin and quercetin-β-3-O-glucoside with minor amounts of luteolin. These flavonoids make up about 2% of the hindwing live weight and are also abundant in the yellow hindwings of several related species of band-winged grasshoppers. Fat body UDPG glucosyltransferase preferentially catalyzed glucosylation of the 3-OH of quercetin.
Key words. Cuticle; flavonoids; glucosides; glucosyltransferase; grasshoppers; quercetin; pigments.

The band-winged grasshoppers (Oedipodinae) characteristically have hindwings brightly pigmented with various shades of yellow, orange, red, or blue [1]. Black bands often outline the inner colored areas of the hindwings. However, the Carolina grasshopper, *Dissosteira carolina*, has a large inner area of the fan that is black, while the outer band is light yellow. We have carried out a study of the yellow pigments in the hindwings of *D. carolina* and several related species and found them to be composed primarily of the common plant flavonoid, quercetin (3,3′,4′,5,7-pentahydroxyflavone) and a quercetin conjugate, identified as the β-3-O-glucoside.

We initially extracted the yellow hindwing cuticle by grinding it in a glass tissue grinder in ice cold 10% HCl followed by adsorption of *o*-diphenolic compounds on alumina at pH 8.6 [2]. The *o*-diphenols, recovered from alumina in 1 M acetic acid, were analyzed by high pressure liquid chromatography (HPLC) on a 4.6 × 250 mm reverse phase C18 5 µm spherical particle column at a flow rate of 1 ml/min with an electrochemical detector (+ 0.72 V). The primary mobile phase consisted of 26% acetonitrile, 1.1 mM sodium dodecyl sulfate and 0.05 mM disodium EDTA in 0.1 M phosphate buffer at pH 3.3 [3]. Other mobile phases consisted of 15–20% acetonitrile in 0.1 M phosphate buffer pH 3. Two major unknown electroactive compounds behaving as *o*-diphenols, were observed with retention times of 7.1 and 26.9 min in the primary mobile phase (fig. 1). After an aliquot of the extract in 10% HCl was heated for 10 min at 100 °C, the 7-min peak disappeared, indicating that it was an acid labile conjugate. Additional extracts of yellow wing cuticle were prepared by grinding the samples in 100% methanol followed by paper chromatography with a mobile phase of n-butanol, acetic acid and water [4]. A yellow band, characteristic of certain flavonoids, was observed under ultraviolet light after exposure to ammonia fumes. This material was extracted from the paper with methanol and gave two peaks by HPLC analysis with retention times identical to those for the *o*-diphenolic compounds previously recovered by alumina adsorption. To identify the free compound, a large sample of yellow wing cuticle was extracted in methanol which was evaporated under nitrogen. The extract was then heated in 10% HCl for 10 min at 100 °C to hydrolyze the conjugate and cooled overnight at 5 °C. A yellow pigment precipitated and was collected by centrifugation and washed with distilled water. The pigment was dissolved in 100% methanol and gave an absorbance spectrum identical to that of quercetin (fig. 2). Absorption maxima were observed at 255, 270 shoulder, and 372 nm. The yellow pigment also had a retention time identical to that of standard quercetin (fig. 3), and the two compounds

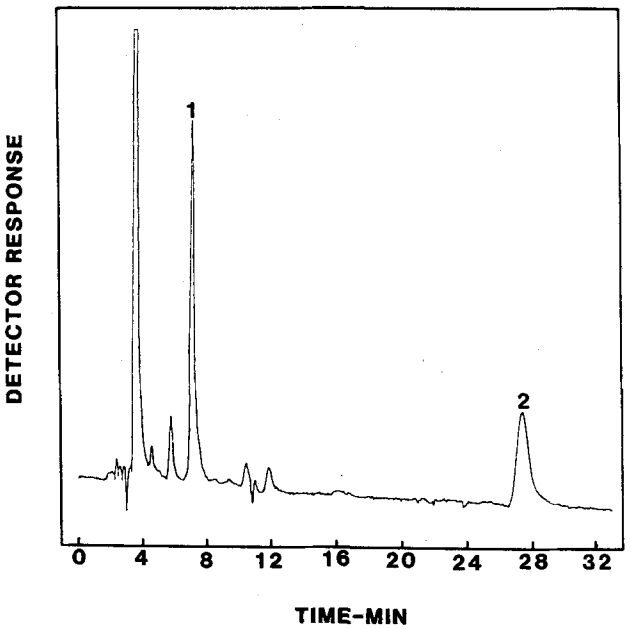

Figure 1. HPLC with electrochemical detection of compounds extracted from the yellow hindwing cuticle of the grasshopper, *Dissoteira carolina*. Peak 1 had the same retention time as the quercetin-3-O-glucoside standard and peak 2 the same as the quercetin standard.

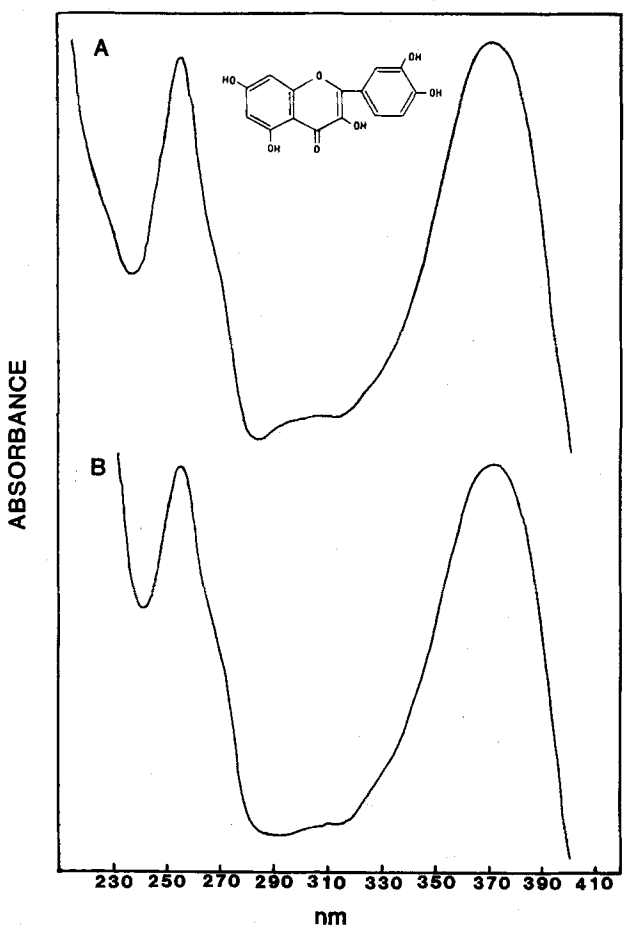

Figure 2. Absorbance spectra of *A*. quercetin standard *B*. flavonoid aglycone isolated from the yellow hindwing cuticle of the grasshopper, *Dissosteira carolina*.

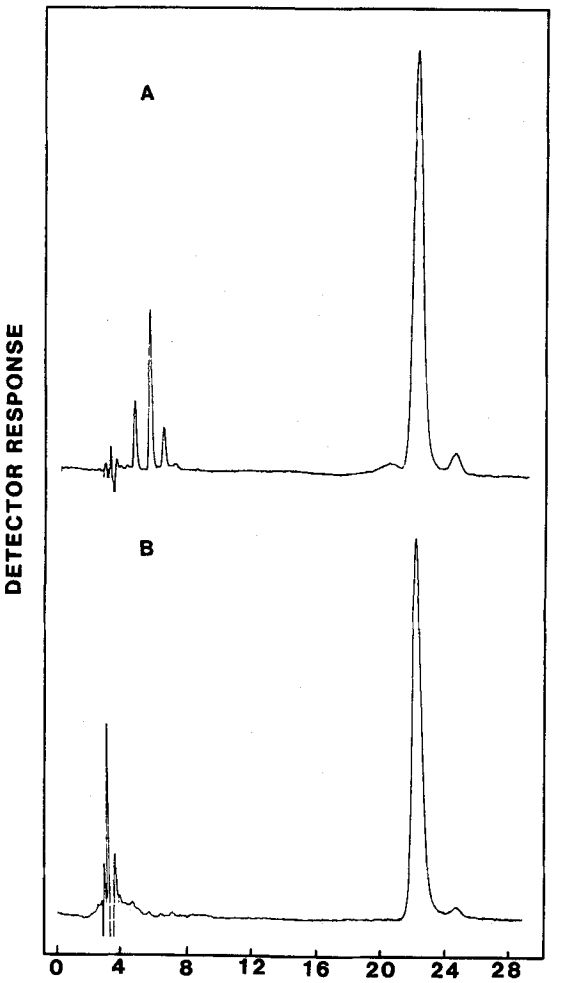

Figure 3. HPLC with electrochemical detection of *A*. quercetin standard *B*. flavonoid aglycone isolated from the yellow wing cuticle of the grasshopper, *Dissoteira carolina*. The small peak eluting after 24 min in both the standard and isolated quercetin had a retention time identical with that of luteolin.

coeluted as symmetrical peaks when analyzed by two different HPLC mobile phases. Small amounts of a compound eluting about 2 min after quercetin (fig. 3) had a retention time identical to that of standard luteolin (3′,4′,5,7-tetrahydroxyflavone).

A sample of the purified yellow pigment when analyzed by probe desorption mass spectrometry gave a molecular ion of 302 of major intensity corresponding to the quercetin standard. A less intense molecular ion of 286 was also observed corresponding to luteolin.

The conjugate was isolated by HPLC with an acetonitrile-phosphate buffer mobile phase and gave an absorbance spectrum characteristic of quercetin glycosylated at the 3-O-position with a shift of the 372 nm absorbance peak to 355 nm[4]. Samples of the isolated conjugate were hydrolyzed in 10% HCl at 100 °C for 10 min. Sugar analysis by capillary gas chromatography with flame ionization detection (GC-FID) of the acid-hy-

drolyzed conjugate showed only glucose to be present in the sample. Quercetin was the only product detected in the hydrolysate when aliquots were analyzed by HPLC. Aliquots were dried under a stream of nitrogen with warming and derivatized in equal parts of *bis* (trimethylsilyl)-trifluoroacetamide (BSTFA) containing 1% trimethylchlorosilane (TMCS) and pyridine at 90 °C for 30 min. The samples and derivatized standards of α and β-D-glucose, D-galactose, D-mannose, α-L-rhamnose, and β-D-fructose (Sigma Chemical Co., St. Louis, MO) were analyzed by GC-FID. The column was an SGE BP-1 0.33 mm ID × 25 m with a nitrogen carrier gas head pressure of 6 psi and a temperature program of 190 °C (2 min) to 240 °C (5 min) at 5 °C/min. The retention times of 11.5 and 13.0 min in the quercetin conjugate sample corresponded exactly with those of the glucose standards but not of any of the other sugars. The conjugate had retention times by two HPLC systems identical to those of standard quercetin-3-O-glucoside (Spectrum Chem. Co., Gardena, CA) and coeluted with it. The absorbance spectrum of the conjugate also matched that of standard quercetin-3-O-glucoside, with absorption maxima at 255, 265 shoulder, and 355 nm. Incubation of the conjugate with almond β-glucosidase in 0.1 M sodium acetate buffer (pH 5) at 37 °C with shaking for 3 h resulted in the disappearance of the conjugate, indicating that it was a β-glucoside.

Analysis of the inner black areas of the hindwing also revealed relatively high concentrations of quercetin and quercetin-β-3-O-glucoside. The black pigmentation patterns, therefore, mask the yellow pigmentation in a species-specific pattern. The concentration of quercetin and its glucoside made up approximately 2% of the live weight of the hindwing cuticle.

Yellow wing cuticle was also extracted and analyzed from the related oedipodine grasshoppers, *Arphia simplex* (Scudder), *Hadrotettix trifaciatus* (Say), *Hippiscus ocelote* (Saussure), *Pardalophora haldemani* (Scudder), and *Spharagemon equale* (Say). Extracts of all species contained relatively large amounts of compounds with the retention times of quercetin and its β-3-O-glucoside, suggesting that these flavonoids are commonly found as yellow pigments in the band-winged grasshoppers.

Flavonoids of plant origin have been found previously as pigments in butterfly wings[5,6]. Those studies have revealed that the flavonoids in the food plants closely corresponded with the aglycones in the wings and bodies of the adult butterflies[7]. The flavonoids are sequestered during larval feeding and are deposited in species-specific patterns in the wings. The same may also apply to the oedipodine grasshoppers with yellow pigmented wings. Experiments in our laboratory show that both fat body and gut tissues are able to glucosylate quercetin. Fat body and mid and hindgut tissues from an adult female *D. carolina* were homogenized in 0.1 M phosphate buffer (pH 7). Aliquots of the tissue homogenates were incubated with quercetin (2.5 mM), uridine diphosphoglucose (UDPG, 5 mM), and $MgCl_2$ (25 mM) in a total volume of 0.2 ml of phosphate buffer for 2 h at 30 °C. The reaction was stopped by adding 0.8 ml of cold methanol. The incubations were centrifuged, and the supernatant was analyzed by HPLC with an absorbance detector at 357 nm. The fat body preferentially synthesized the β-3-O-glucoside with minor amounts of three other unidentified products, that behaved as glucosides. These products eluted earlier than quercetin-3-O-glucoside. The gut synthesized quercetin-3-O-glucoside but also a nearly equal amount of one of the unidentified conjugates and lesser quantities of the other two. The fat body tissue was also more active than the gut in synthesizing quercetin-3-O-glucoside (1.8 vs 0.056 nmol/min/mg protein, respectively). UDPG was essential for the formation of quercetin glucosides by these tissues. Our studies also showed that the gut and fat body tissues contain β-glucosidases, but we have not studied these in detail. Because quercetin in plant tissues is largely conjugated with glucose, rhamnose, and other sugars, it is possible that quercetin glycosides in the diet are hydrolyzed during absorption through the gut. Glucosylation on the 3-hydroxyl position occurs most specifically in the fat body, which may account for the predominance of this conjugate in the wings after transport in the hemolymph. Flavonoids have not been reported previously as grasshopper pigments. Our results raise some interesting questions concerning the sequestration, processing, and transport of flavonoids into the wings of grasshoppers for pigmentation and possibly other functions.

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1 Otte, D., The North American Grasshoppers, Acrididae, Oedipodinae, vol. II. Harvard Univ., Cambridge, MA 1984.
2 Murdock, L. L., and Omar, D., Insect Biochem. *11* (1981) 161.
3 Morgan, T. D., Hopkins, T. L., Kramer, K. J., Roseland, C. R., Czapla, T. H., Tomer, K. B., and Crow, F. W., Insect Biochem. *17* (1987) 255.
4 Mabry, T. J., Markham, K. R., and Thomas, M. B., The Systematic Identification of Flavonoids. Springer-Verlag, New York 1970.
5 Morris, S. J., and Thomson, R. H., J. Insect Physiol. *9* (1963) 391.
6 Wilson, A., Phytochemistry *24* (1985) 1685.
7 Wilson, A., J. chem. Ecol. *11* (1985) 1161.

0014-4754/91/101089-03$1.50 + 0.20/0