3,7-Di-*O*-methylquercetin 5-*O*-Glucoside from *Zea mays*

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A novel flavonol glycoside, identified as 3,7-di-*O*-methylquercetin 5-*O*-glucoside, was isolated from aqueous homogenates of corn *Zea mays* whorl tissue. The related aglycon was also identified but was less abundant. Whorl tissue from insect resistant and susceptible corn hybrids contained similar amounts of the glucoside.

INTRODUCTION

Although flavonol glycosides and other glycosides have a limited occurrence in corn *Zea mays* L., DIMBOA glucoside (4-*O*-glucosyl-2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one) has been reported as a major basis of resistance to the European corn borer *Ostrinia nubilalis* (Hubner) (Klun and Brindley, 1966). Another flavonoid, maysin (luteolin 6-rhamnosyl-4-ketofucoside), was isolated from corn silks and reported to have antibiotic activity toward the corn earworm *Heliothis zea* (Boddie) (Waiss et al., 1979). In the course of our studies with selected corn lines (Williams et al., 1989) differing in resistance to fall armyworm *Spodoptera frugiperda* (J. E. Smith) and southwestern corn borer *Diatraea grandiosella* Dyar, we detected both of these glycosides; however, they were present in both susceptible and resistant plants at concentrations too low to be significant factors of resistance (Hedin et al., 1984). Recently we observed yellow pigments in aqueous homogenates of whorls from these corn lines following removal of all chlorophyllous and other cellular membranes by centrifugation at 100000*g*. In our previous work (Hedin et al., 1984), typical organic extractions of corn whorl tissues did not reveal such a pigment. Nasser et al. (1988) also noted, but did not identify, yellow pigments associated with aqueous protein extracts of corn leaves.

Here we report a structural characterization of this yellow pigment and, further, compare corn lines with well-documented differences in susceptibility to fall armyworm and southwestern corn borer for the presence of the compound.

MATERIALS AND METHODS

Plant Material. Susceptible (S) hybrid, Ab24E X Tx601, and a mixture of two resistant (R) hybrids, Mp703 X Mp707 and Mp704 X Mp707, were used in this study. These lines were developed, grown under field conditions, and provided to us by the Corn Host Plant Research Unit (USDA-ARS) at Mississippi State, MS (Williams et al., 1989). Corn whorl tissue was collected from plants grown to the V8–V10 stage of development (Ritchie and Hanway, 1982) and was stored sealed in plastic bags at −20 °C until extractions were done.

Extraction. Approximately 1200 g each of the S and R whorls was homogenized at 4 °C in separate 300-g batches upon addition of 900 mL of extraction buffer (10% glycerol, 0.1 M HEPES, 1 mM EDTA, 2 mM sodium ascorbate, 1 mM DTT, 0.4 mM PMSF, final pH 7.5). Duration of grinding was 2–3 min or until a slurry was obtained. The homogenate was filtered through two layers of Mira-cloth (Calbiochem) and then centrifuged at 10000*g* for 45 min in a GSA rotor (Sorvall). The resulting supernatant was then centrifuged at 100000*g* for 1 h in a SW28 rotor (Beckman). The resulting yellow supernatant was immediately fractionated as below. We have since discovered that filtering the 10000*g* supernatants through acid-washed diatomaceous earth (Celite) clarifies the sample equivalent to the ultracentrifugation step with regard to removal of any remaining cellular membranes.

Fractionation. The final 100000*g* supernatants of each grind were separated from protein by chromatography (4 °C) on Sephadex G-25 (50–150 mesh) columns (5 × 70 cm) preequilibrated with water; elution was with water. Protein eluted after the void volume (600–800 mL), while the yellow pigment eluted at 1400–1500 mL and was collected in a 150-mL fraction. The eluate was lyophilized to yield a gummy solid. The final yields from S and R tissues were 0.31 and 0.42 g, respectively.

Aliquots of these solids were dissolved in MeOH and subjected to polyamide TLC with MeOH as the solvent. Products were visualized with 1% ethanolic diphenylboric acid 2-aminoethyl ester under ultraviolet light. The main compound fluoresced blue at R_f = 0.50, indicative of a monoglycoside. A minor band that fluoresced tan at R_f = 0.05 was also observed. No differences were noted between S and R isolates.

The remaining lyophilized solids were dissolved in MeOH/H_2O (3:1) and chromatographed at room temperature on Sephadex LH-20 (25–100 mesh) columns (3 × 30 cm) by using the same solvent mixture. Two fractions occurring again in both S and R samples were collected, a faster migrating yellow-orange fraction (A) and a later eluting orange-brown fraction (B). Examination of these two fractions by the aforementioned polyamide TLC revealed that A (R_f = 0.5) and B (R_f = 0.05) were the blue and tan fluorescing compounds, respectively. Lyophilization of these two fractions from S and R samples resulted in the following yields: yellow-orange fraction A, 0.16 (S) and 0.21 g (R); orange-brown fraction B, 0.08 (S) and 0.11 g (R). Thus, elution volumes, R_f values, and overall yields of the yellow pigments were essentially the same for S and R whorl tissue.

Acid Hydrolysis. Portions (ca. 160 mg) from both the S and R sources of fraction A were hydrolyzed by refluxing for 1 h in an aqueous MeOH (4:1) solution made to 1 N HCl. Water was then added to the reflux mixture, and the cooled solution was extracted with EtOAc to separate the resulting aglycon. The H_2O layer was evaporated to dryness, and the residue containing any released sugars was dissolved in pyridine. Yields of aglycon and sugar were 0.095 and 0.061 g, respectively, indicative of a 1:1 ratio.

Ultraviolet and Visible Spectra. Spectra were collected with a Perkin-Elmer Lambda 4B spectrophotometer. Spectral data were obtained in MeOH, and spectral shifts were examined after the addition of aluminum chloride, sodium methoxide, and sodium acetate (Mabry et al., 1970).

^{1}H NMR Spectra. Spectra were obtained on the aglycon in acetone-d_6 and on the TMSi glycoside in $CDCl_3$ with a QE-300 GE NMR spectrometer (Mabry et al., 1970). The TMSi ether

Table I. Spectral Data Obtained on Sephadex LH-20 Eluate Fractions A and B (Glycoside and Aglycon) from Corn Whorl Aqueous Tissue Extracts

	A		B	
UV–vis				
spectral band	II	I	II	I
methanol	272 (299 sh)	320	270	345
sodium acetate	275	323	273	341
sodium methoxide	284	373	274	407
aluminum chloride	280 (306 sh)	326	279	390 (361 sh)

	A	B
^{1}H NMR, ppm		
GL protons (m)	3.48, 3.57	
OCH_3-3,7 (s)	3.85	3.97
H-1 (GL) (s)	4.90	
H-6 (s)	6.23	6.27
H-8 (s)	6.62	6.57
H-5′ (s)	6.82	6.74
H-2′, H-6′ (s)	7.33, 7.40	7.35, 7.39
mass spectra, m/z		
free aglycon (EIMS)		330 $[M]^+$, 300, 213, 194, 177, 164, 163, 91
hydrolysis product (EIMS)		330 $[M]^+$, 300, 213, 194, 177
sugar residue (CIMS)		163 [M + 1 − 18], 145

of the flavonol glycoside was prepared by adding hexamethyldisilazine and trimethylchlorosilane to 50 mg of the glycoside that had been dissolved in dry pyridine.

Mass Spectra. Aglycons were analyzed via solid probe (70 eV) in the EIMS mode with a Hewlett-Packard 5985-B mass spectrometer. The sugar residue released by acid hydrolysis was analyzed via solid probe in the CIMS (methane) mode.

RESULTS AND DISCUSSION

Eluate fractions A and B from Sephadex LH-20 chromatography (Materials and Methods) were subjected to acid hydrolysis and reanalyzed by polyamide TLC. Hydrolyzed fraction A migrated with the same R_f (0.05) as original fraction B, while fraction B was not affected by hydrolysis. Mass spectra (EIMS) of hydrolyzed fractions A and B gave the same M^+ 330 (deduced to be $C_{17}H_{14}O_7$) with nearly identical fragmentation patterns (Table I). Conversely, the residue from the aqueous phase of the acid hydrolysis step gave a fragmentation pattern (CIMS, CH_4) consistent with that of glucose (Table I). We conclude from these data that fraction B is the aglycon of fraction A. The yields of aglycon and sugar upon hydrolysis and the TLC R_f of the glycoside were consistent with those of a monoglycoside.

UV–visible spectra (Table I) of the aglycon (B) and the hydrolyzed product from fraction A showed that the addition of sodium methoxide gave a band I bathochromic shift of 61 nm, suggestive but not mandatory for a free 4′-OH. The band I bathochromic shift with aluminum chloride was 45 nm, indicative of a free 5-OH and a protected 3-OH. If the 3-OH were also free, a shift of approximately 60 nm would have been observed. The absence of a 10-nm band II bathochromic shift upon the addition of sodium acetate indicated that the 3-, 7-, and/or 4′-OH was missing or protected. Because a shift occurs on addition of sodium methoxide, the presence of a free OH at the 4′-position, but not the 3- or 7-position, is inferred.

The UV–visible spectrum of fraction A showed a hypsochromic shift of 25 nm for the glycoside relative to that of the band I absorption of the aglycon (B). The only other significant spectral change of glycoside A relative to aglycon B is the absence of a bathochromic shift upon the addition of aluminum chloride, suggesting that the 5-OH is glycosidated, given that the 3-OH of the aglycon was shown to be absent or protected.

^{1}H NMR data for fraction B (Table I) accounted for two methoxy groups and protons at 6-H, 8-H, 2′-H, 5′-H, and 6′-H′. ^{1}H NMR data for fraction A accounted for the glucose protons and the H-1 glucose, in addition to those of aglycon B. The ^{1}H NMR data are consistent with the presumptive requirement for two methoxy groups given the M^+ 330 with the elemental formula $C_{17}H_{14}O_7$.

On the basis of these data, we assign the structure of this corn pigment as 3,7-di-*O*-methylquercetin 5-*O*-glucoside. Glycosidation at the 5-position is apparently quite rare, although at least four flavone or flavonol glycosides, sakuranin, luteolin 5-glucoside, chrysin 5-glucoside, and genkwanin 5-glucoside, are glucosidated at this position (Hattori, 1962; Mabry et al., 1970). However, to our knowledge this specific compound has not been previously reported. The relatively low polarity of the isolated aglycon (B) makes its presence in these aqueous homogenates somewhat unexpected; the possibility of artifactual formation of the aglycon during isolation procedures was not ruled out. We also obtained evidence for traces of tri- and tetra-*O*-methylquercetins based on apparent molecular ions at m/z 342 and 356 in the ethyl acetate extract of the acid hydrolysate of the total pigment fraction (data not shown).

While not present in greater concentrations in the insect-resistant corn whorls (Materials and Methods), the flavonol glycoside reported here with its 3′-OH,4′-OH functionality may possess some toxicity for insects. Elliger et al. (1980), in a study comparing antigrowth activity of 40 flavonoids, reported that orthodihydroxylation in either A or B ring was necessary for growth inhibition of *H. zea*. The orthodihydroxylation function was also found necessary for antibacterial activity (Hedin and Waage, 1986).

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