Phytochemistry, Vol. 23, No. 8, pp. 1822–1823, 1984.
Printed in Great Britain.

0031–9422/84 $3.00 + 0.00

FLAVONOIDS FROM *ZEA MAYS* POLLEN

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(*Revised received* 22 *January* 1984)

Key Word Index—*Zea mays*; Poaceae; maize; pollen flavonoids; flavonol glycosides.

Abstract—The flavonol glycosides of quercetin, isorhamnetin and kaempferol were isolated from *Zea mays* pollen. The most prominent flavonols were diglycosides of quercetin and isorhamnetin. Flavonol 3-*O*-glucosides of quercetin, isorhamnetin and kaempferol, and triglucosides of quercetin and isorhamnetin, were minor components. The flavonoid pattern of maize pollen is characterized by the accumulation of quercetin and isorhamnetin diglycosides and by the absence of flavones, which are common in other maize tissues.

INTRODUCTION

Little has been published on the flavonoid derivatives from maize (*Zea mays* L.) pollen. Redeman *et al.* [1] identified the yellow pigment in an ether extract of maize pollen as quercetin, and Larson and Coe [2] reported that the maize pollen of most genotypes contains quercetin and isoquercitrin (quercetin 3-glucoside). Wiermann [3] found quercetin, isorhamnetin and kaempferol in a hydrolysed extract of maize pollen. This paper presents a more detailed description of the flavonoid glycosides isolated from maize pollen.

RESULTS AND DISCUSSION

Ten flavonol glycosides of quercetin, isorhamnetin and kaempferol were isolated from *Zea mays* pollen. They are the 3-*O*-glucosides of quercetin (**8**), isorhamnetin (**9**) and kaempferol (**10**); quercetin 3,3′-*O*-diglucoside (**2**), quercetin 3,7-*O*-diglucoside (**1**) and quercetin 3-*O*-neohesperidoside (**4**); isorhamnetin 3,4′-*O*-diglucoside (**3**) and isorhamnetin 3-*O*-neohesperidoside (**5**). The triglucosides quercetin 3-*O*-glucoside-3′-*O*-diglucoside (**6**) and isorhamnetin 3-*O*-glucoside-4′-*O*-diglucoside (**7**) were also present. Five of the compounds (**1**–**5**) were accumulated in sufficient quantity for NMR spectral analysis. The remaining five (**6**–**10**) were identified by their UV spectral properties, R_f values, colour reactions, and by comparison with known compounds. All ten were glycosides of kaempferol, quercetin, or isorhamnetin. Several other compounds were seen in trace amounts only and were not considered further. R_f values for identified flavonols are presented in Table 1.

The most prominent compounds in the profile (**1**–**3**) behaved as diglycosides. The total acid hydrolysis of **1** and **2** yielded quercetin and glucose, while that of **3** yielded isorhamnetin and glucose. UV spectral data showed **1** to be substituted at positions 3 and 7. Partial hydrolysis and ^{1}H NMR analysis supported the identification as quercetin 3,7-*O*-diglucoside. UV spectral data suggested **2** to be quercetin with substituents at positions 3 and 3′. Since acid hydrolysis yielded only glucose, this compound was identified as quercetin 3,3′-*O*-diglucoside. Compound **3** was in all respects (UV and NMR spectra, hydrolysis

Table 1. R_f values for flavonol glycosides of *Zea mays* pollen

Compounds*	Relative† amount	R_f (× 100)‡ (1)	(2)	(3)	(4)
Km 3-*O*-glucoside	trace		73		38
Qu 3-*O*-glucoside	+	53	55	06	27
Qu 3,7-*O*-diglucoside	+ + +	14	19	28	62
Qu 3,3′-*O*-diglucoside	+ + + + +	25	24	19	59
Qu 3-*O*-neohesperidoside	+ +	51	49	41	75
Qu 3-*O*-glucoside-3′-*O*-diglucoside	trace	12	10	32	68
Ir 3-*O*-glucoside	trace	62	70	05	31
Ir 3,4′-*O*-diglucoside	+ +	27	28	26	65
Ir 3-*O*-neohesperidoside	+	57	56	44	78
Ir 3-*O*-glucoside-4′-*O*-diglucoside	trace	12	16	47	75

*Km = kaempferol, Qu = quercetin, Ir = isorhamnetin.

†Estimated by visual examination of TLC plates.

‡Solvent systems: (1) TBA; (2) EtOAc–HCOOH–H_2O (10:2:3); (3) H_2O; (4) 15% HOAc. Avicel microcrystalline cellulose was used in all cases.

products) identical with isorhamnetin 3,4′-*O*-diglucoside (dactylin). Compounds **1–3** were subjected to enzymic hydrolysis by β-glucosidase and the only intermediate detected was the 3-glucoside. Partial acid hydrolysis of these compounds gave quercetin 7-glucoside, quercetin 3′-glucoside and isorhamnetin 4′-glucoside, respectively. This is in agreement with findings published by Harborne [4].

In aqueous solvents **4** and **5** also behaved as diglycosides. The total acid hydrolysis of **4** yielded quercetin, glucose and rhamnose, and that of **5** isorhamnetin, glucose and rhamnose. The UV spectral data for **4** were similar to that of rutin but the R_f values were different. The ^{1}H NMR spectra of **4** and **5**, showing the presence of a 3 proton doublet at δ0.88–0.92 confirmed that rhamnose was one of the sugars. In addition, a 1 → 2 interglycosidic linkage was indicated for each compound by the presence of singlets at 4.75 for **4** and 4.72 for **5** (attributed to the rhamnose H-1 [5]) and glucose H-1 proton signals at δ5.75. In each case hydrogen peroxide oxidation produced a disaccharide which was identical on co-chromatography with authentic neohesperidose (prepared from natural naringenin 7-neohesperidoside). Thus, **4** and **5** were assigned to the 3-*O*-neohesperidosides of quercetin and isorhamnetin. Isorhamnetin 3-neohesperidoside has been previously found in *Nerisyrenia* [6].

Compounds **6** and **7** behaved as triglycosides. Total acid hydrolysis yielded quercetin from **6**; isorhamnetin from **7** and only glucose in both cases. The UV spectral data of **6** suggested substituents at positions 3 and 3′. Partial hydrolysis of **6** gave quercetin 3′-glucoside and a diglucoside (likely to be quercetin 3′-diglucoside), and partial hydrolysis of **7** gave isorhamnetin 4′-glucoside and a diglucoside (likely to be isorhamnetin 4′-diglucoside). Compound **6** is therefore quercetin 3-*O*-glucoside-3′-*O*-diglucoside and **7** is isorhamnetin 3-*O*-glucoside-4′-*O*-diglucoside. Because only small amounts of these compounds were present it was not possible to establish interglycosidic linkages.

Three other minor compounds were shown to be 3-glucosides of quercetin (**8**), isorhamnetin (**9**) and kaempferol (**10**). Compounds **8** and **9** were identified by co-chromatography with corresponding glycosides found in maize plant tissues [7]. Kaempferol 3-glucoside is present only in traces in both pollen and plant tissues and was identified on the basis of R_f values, colour reactions, and comparison with a standard.

The major identified flavonol glycosides (**1–5**) may be pollen specific [8]. Pratviel-Sosa [9] also found pollen specific flavonol glycosides in the pollen of some trees. The flavonoid pattern of maize pollen is also characterized by the complete absence of flavones, which are otherwise common in most maize tissues. In maize, the majority of the genes known to affect the flavonoid pattern of the plant and seed have no effect on the pollen glycosides. Exceptions are *bz* stocks, where concentrations of 3-*O*-glycosylated flavonols are considerably reduced and glycosides with a free 3-hydroxyl group accumulate [8].

EXPERIMENTAL

Plant material. Tassels (from *Bz* stocks, see text) were collected from flowering plants in the field and air dried in the lab. Pollen was separated from anthers by sieving through a Tylor test sieve with 125 μm openings.

Extraction and chromatography. Dried pollen (approximately 300 g) was extracted using boiling 70% MeOH. The extract was concd under red. pres., put in boiling water for treatment with Celite Analytical Filter Aid, filtered under a vacuum and then extracted with EtOAc [10]. An additional extraction of the aq. extract with *n*-BuOH improved the yield of the polar flavonoids [11]. Initial separation was made on a Sephadex LH-20 column, using a linear elution gradient of increasing concentration of MeOH in H_2O. Column fractions were repeatedly chromatographed on Whatman No. 3 chromatographic paper using EtOAc–HCOOH–H_2O (10:2:3) or 15% HOAc. The final purification of compounds was achieved by repeated TLC on Polyamide DC-6.6, using H_2O–*n*-BuOH–Me_2CO–HOAc (16:2:1:1).

Hydrolysis. Acid hydrolysis was performed using TFA at 80–100° for 1 hr. The hydrolysis of diglycosides was monitored by TLC at 10–20 min intervals [12]. H_2O_2 oxidation was conducted following Harborne's [13] procedure. Enzymic hydrolysis was done according to Markham [14].

Identification of hydrolysis products. Aglycones were identified by co-chromatography with standards on Polyamide DC-6.6 TLC using $CHCl_3$–MeCOEt–MeOH (14:3:3) and colour after spraying with 0.1% diphenylboric acid aminoethyl ester in MeOH–H_2O (1:1) and also by co-chromatography on microcryst. cellulose TLC using EtOAc–HCOOH–H_2O (10:2:3). Sugars were chromatographed either on cellulose TLC in EtOAc–pyridine–H_2O (10:3:2) and detected by spraying with the reagent described by Mabry *et al.* [5], or on precoated silica gel 60 (without fluorescent indicator) plastic sheets using $CHCl_3$–MeOH–H_2O (16:9:2) and detected by spraying with 0.5 g thymol in 95 ml EtOH and 5 ml of conc. H_2SO_4 [10].

UV and ^{1}H *NMR.* UV analysis was performed according to Mabry *et al.* [5]. ^{1}H NMR spectra were performed on per-trimethylsilyl derivatives with TMS as an int. standard.

Acknowledgements—We would like to thank the National Research Council of Canada for its financial support, and Dr. B. Bohm for his help with NMR analysis.

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