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Full Length Research Paper

Isolation and identification of two novel flavone glycosides from corn silk (*Stigma maydis*)

Shun-Cheng Ren[1*], Ze-Long Liu[2] and Xiao-Lin Ding[2]

[1]School of Food Science and Technology, Henan University of Technology, Zhengzhou 450052, China.
[2]School of Food Science and Technology, Jiangnan University, Wuxi 214122, China.

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Corn silk has been found to be an excellent source of bioactive compound. Two novel flavone glycosides of corn silk were identified by means of spectral analyses as 2″-O-α-L-rhamnosyl-6-C-3″-deoxyglucosyl-3′-methoxyluteolin and 6,4′-dihydroxy- 3′-methoxyflavone -7-O-glucoside. Also, the flavonoid contents of 40 corn silk lines collected from 16 provinces in China were analyzed by high performance liquid chromatography and colorimetric methods.

Key words: Corn silk, flavonoids, isolation, chromatography, spectral analyses.

INTRODUCTION

Corn silk is made up of the stigmas and styles of the maize plant belonging to the grass (*Gramineae*) family. Corn silk has been used in traditional Chinese medicine for the treatment of hypertension, hepatitis, tumor, hyperglycemia, etc (Li et al., 1995; Liu, 1995; Ma et al., 1998). It also possesses immune enhancing effects, as well as diuretic, cholagogic and demulcent functions (Tang et al., 1995; Namba, 1993). As a soothing diuretic, it is mainly used clinically for the treatment of urethritis, cystitis, nephritis, lithiasis (urinary stone), gonorrhoea and prostatitis in China (Wang, 1991).

It has been found that corn silk is an excellent source of many bioactive compounds such as flavonoids, saponin, alkaloids, tannins, chlorogenic acid, phytosterols, allantoin, vitamin E and K, etc (Bushman, 2002). To date, several flavonoids, such as maysin, apigmaysin, 3′-methoxymaysine, ax-4″-OH-maysin, etc, have been isolated and identified from corn silk (Waiss et al., 1979; Elliger et al., 1980; Snook et al., 1995). Nevertheless, the multiple functions of corn silk suggest the possible presence of more previously unidentified compounds. In this regard, the present study was carried out to discover the flavonoid compounds in corn silk that may relate to its beneficial effects on human health.

*Corresponding author. E-mail: scren@163.com. Tel: 371–68883238. Fax: 371–67789817.

MATERIALS AND METHODS

General

UV spectra were measured on a Shimadzu UV 1601. Mass spectra were collected on a Micromass ZMD LC/MS spectrometer operated in ESI negative mode. The ^{1}H, ^{13}C, DEPT and HMQC nuclear magnetic resonance (NMR) spectra were obtained on a Bruker Avanced 500 MHz NMR spectrometer, using standard pulse sequences. Chemical shifts were reported on the δ scale in parts per million downfield from TMS. TLC was carried out on a precoatedf silica gel 60 F_{254} plates (Merck), developed with chloroform-methanol-water-acetic acid (70:30:10:1, v/v). Chromatography was performed on silica gel column (Marine Chemical Factory in Qingdao) and Toyopearl HW-40 (TOSOH).

Plant material

Air-dried corn silk was collected from 16 provinces across China in 2004. The corn silk was crushed and stored in a sealed container at below 8℃ until used.

Extraction and isolation

A total of 8 kg of corn silk powder was divided into 4 portions, then each portion (2 kg) was extracted 3 times at room temperature using 5 L of 80% v/v ethanol/water (3×5 L). The three extracts of each portion were combined and concentrated to 1.5 L (745 g/dry weight) at 45℃ under reduced pressure using rotary evaporator, then washed three times with 500 mL of petroleum ether and then extracted three times using 500 mL of ethyl acetate. These three extractions of each portion were then combined and concentrated

to 160 mL (4.8 g/dry weight) at 45℃ under reduced pressure.

Aliquots of 100 mL of water were added to the above solutions and the resulting mixtures were fractioned using polyamide column chromatography (100 mesh, 40×600mm), eluted with ethanol-water (0 to 60%) and monitored by thin layer chromatography (TLC) performed on GF_{254} silica gel plates. Four fractions per portion were collected (1203，505，146，34，87 mg/ dry weight, respectively) and independently further fractionated on a Toyopearl HW-40 column (35×500 mm) with 10, 20, 30 and 40% ethanol, respectively, to give compound I–V (567、232、48、19、32mg/dry weight, respectively) in >95% purity as analyzed by HPLC.

HPLC and colorimetric analysis

Corn silk powder (4 g) was extracted with 80% methanol (100 mL) at 85℃ for 6 h in a Soxhlet extractor. The extracts were then cooled to room temperature, filtered, and methanol was added to adjust the volume to 100 mL. A 20 µL aliquot of each extract filtered through a 0.45 µm membrane was then analyzed for compound I–V by reversed phase HPLC (Waters 2690) with a linear gradient from methanol-water (20:80) to (100%) methanol anhydrous containing 1% ortho-phosphoric acid in 50 min at 1 mL/min, with Waters 2487 UV-Vis detector at 350 nm (detection wavelength) and 550 nm (reference wavelength). The place of flavonoids on HPLC could be defined through the comparison of peak retention time between extracts and standard solution (retention time of compound I–V were 17.7, 21.88, 23.88, 17.08 and 24.00 min, respectively). The content of flavonoids could be calculated through regression equation between peak area and flavonoids content. The total flavonoid content was analyzed by colorimetric assay at 400 nm as described previously (Ren et al., 2003).

Statistical analysis

Data of the flavonoid contents of 40 corn silk lines were subjected to analysis of variance using the general linear model's procedure of the Statistics software 9.0 (Analytical Software, Tallahassee, FL), and Tukey HSD all-pairwise multiple comparisons were performed to identify significant differences between individual corn varieties for the contents of compound I-V.

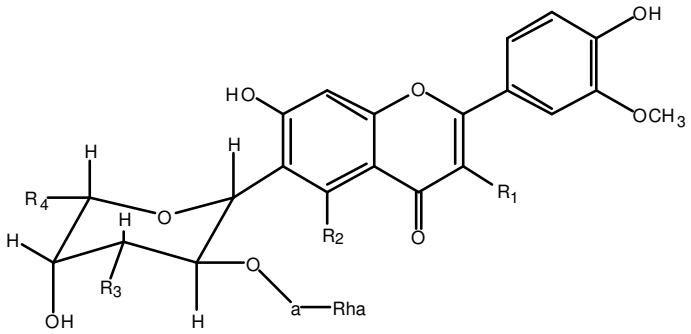
RESULTS AND DISCUSSION

Five flavonoid monomers (compounds I–V) were isolated and identified as the major products of the column separation. Among which II (ax-5″-methane-3′-methoxymaysin), III (ax-4″-OH-3′-methoxymaysin) and V (7,4′-dihydroxy-3′-methoxyflavone-2″-O-α-L-rhamnosyl-6-C-fucoside) have been previously isolated and identified (Ren et al., 2004; Ren et al., 2007) (Figure 1). However, this is the first report for compound I and compound IV from any natural source.

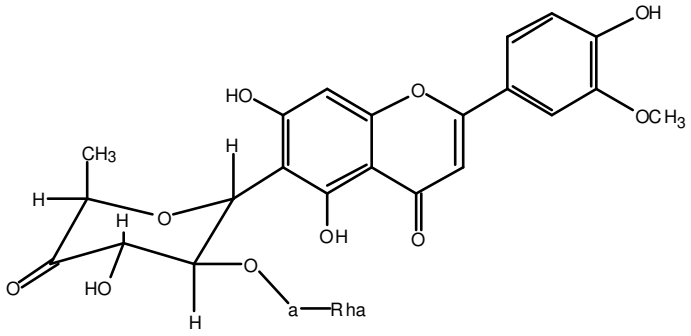
Compound I (2″-O-α-L-rhamnosyl-6-C-3″-deoxyglucosyl-3′-methoxyluteolin) is a grayish white powder, soluble in methanol. When treated with ammonia on thin layer chromatography (TLC) plate, it became yellow. The UV spectrum of compound I showed absorption maxima at 272 and 351 nm in methanol. These results suggested compound I is a flavone derivative. The ESI-MS gave a (M-H) ion peak at *m*/*z* 592, indicating the molecular formula to be $C_{28}H_{32}O_{14}$.

The ^{1}H and ^{13}C NMR spectra showed the presence of a luteolin moiety and two sugar residues moiety (Table 1). Signals at δ 6.98 (1H, s) and δ 6.96 (1H, s) were attributed, respectively, to H-8 and H-3 through the HMQC spectrum, and a singlet at δ 3.83 (3H, s) was assigned to an aromatic methoxyl group at the 3′-position in the B-ring through the HMQC spectrum (Figure 2). Furthermore ABX-type aromatic proton signals at δ 7.51 (2H, s) and δ 6.89 (1H, d, J = 8.4 Hz) in B-ring were observed, and they were attributed, respectively, to H-2′, H-6′ and H-5′ through the HMQC spectrum. The aromatic proton arising from the sugar moiety appeared δ 5.31 (1H, m) and δ 4.91 (1H, m), which correlated with two signals δ 64.7 and δ 102.2 in the HMQC spectrum. These signals were attributed, respectively, to H-1″ and H-1‴. The signals δ 3.0~δ 4.0 were attributed to sugar residues moiety according to the literature values (Elliger et al., 1980; Snook et al., 1995). A signal at δ 102.2 in the ^{13}C NMR spectrum suggested that the compound was not an O-glycoside (Snook et al., 1995) (Figure 3). A signal at δ 64.7 in the ^{13}C NMR spectrum showed the presence of an O-glycoside (Toshihihiro et al., 2001). Signals at δ 60.8 and δ 30.1 were attributed to two CH_2 through the DEPT spectrum (Table 1). A signal at δ 55.9 was attributed to one OCH_3. The signal δ 17.2 was attributed to one CH_3. A signal at δ 102.2 were attributed to C-1‴ (Rhamnose) because of a correlation between δ 4.91 (H-signal) and δ 102.2 (C-signal) in the HMQC spectrum. C-signals of rhamnose were respectively δ 102.2, δ 69.9, δ 70.0, δ 73.7, δ 69.2 and δ 17.2. A signal at δ 64.7 were attributed to C-1″ (glucose) because of a correlation between δ 5.31 (H-signal) and δ 64.7 (C-signal) in the HMQC spectrum. The C-signals of glucose were respectively δ 64.7, δ 75.2, δ 30.1, δ 67.2, δ 77.6 and δ 60.8,among which C-3 was deoxygenated and it linked with δ 2.82 (1H, m) and δ 1.19 (1H, m) through the HMQC spectrum. Rhamnose linked with C-2″ (glucose) because of a correlation between δ 4.91 (H-signal) and δ 75.2 (C-signal) in the HMBC spectrum. According to the above analysis and literature values (Elliger et al.,1980; Snook et al., 1995), compound I was determined to be 2″-O-α-L-rhamnosyl-6-C-3″-deoxyglucosyl-3′-methoxyluteolin.

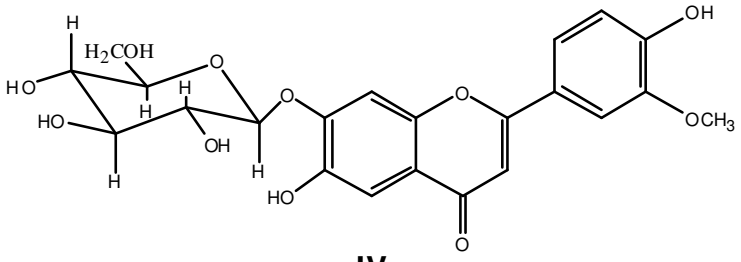
Compound IV (6, 4′-dihydroxy-3′-methoxyflavone-7-O-glucoside) is a yellowish green crystal, soluble in methanol. When treated with ammonia on thin layer chromatography (TLC) plate, it became yellow. The UV spectrum of compound IV showed absorption maxima at 265 and 345 nm in methanol. These results suggest that compound IV is a flavone derivative. The ESI-MS gave a (M+H) ion peak at *m*/*z* 463, indicating the molecular formula to be $C_{22}H_{22}O_{11}$. The (M+H-162) ion peak at *m*/*z* 301 in the ESI-MS indicated that the compound may be a glycoside and the sugar moiety a hexose. Its ^{1}H and ^{13}C NMR spectra also showed the presence of a methoxyflavone moiety (Table 1, Figure 4). Signals at δ 6.81 (1H, s) were attributed to H-3 through the MS spectrum and refer to compound I, and a singlet at δ 3.89



I R_1=H, R_2=OH, R_3=H, R_4=CH_2OH
III R_1=H, R_2=OH, R_3=OH, R_4=CH_3
V R_1=OH, R_2=H, R_3=OH, R_4=CH_3



II



IV

Figure 1. Structures of compound I–V.

(3H, s) was assigned to an aromatic methoxyl group at the 3′-position in the B-ring through the ^{13}C NMR, DEPT spectra and refer to compound I (Table 1). Referring to compound I, proton signals at δ 7.51 (2H, s) and δ 6.89 (1H, d, *J* = 8.4 Hz) in B-ring were observed, and were attributed, respectively, to H-2′, H-6′ and H-5′. Literature values (Angela et al., 1997) as well as the ^{13}C NMR, DEPT and MS spectra suggest the glycosyl residue was glucose. C-signals of glucose were respectively δ 104.5, δ 73.7, δ 75.7, δ 69.7, δ 77.6 and δ 60.9. H-signals of glucose were respectively δ 4.72 (1H, d, *J* = 7.0 Hz, H-1″), δ 3.29 (1H, s-like, H-2″), δ 3.34 (1H, s, H-3″), δ 3.23 (1H, d, *J* = 8.9 Hz, H-4″), δ 3.55 (1H, m, H-5″) and δ 3.89, δ 3.75 (each 1H, s-like, H-6″). A signal at δ 6.75 (2H, s) indicated two hydrogen were p-substituted hydrogen and attributed, respectively, to H-8 and H-5. So the existence of 6-OH was confirmed. According to the above analysis and literature values (Angela et al., 1997), compound IV was determined to be 6, 4′-dihydroxy-3′-methoxyflavone 7-O-glucoside.

In the literature examined, there is little information about the content of those five flavonoid compounds in corn silk. The results of the total flavonoid and flavonoid content analyses for 40 samples of corn silk lines from

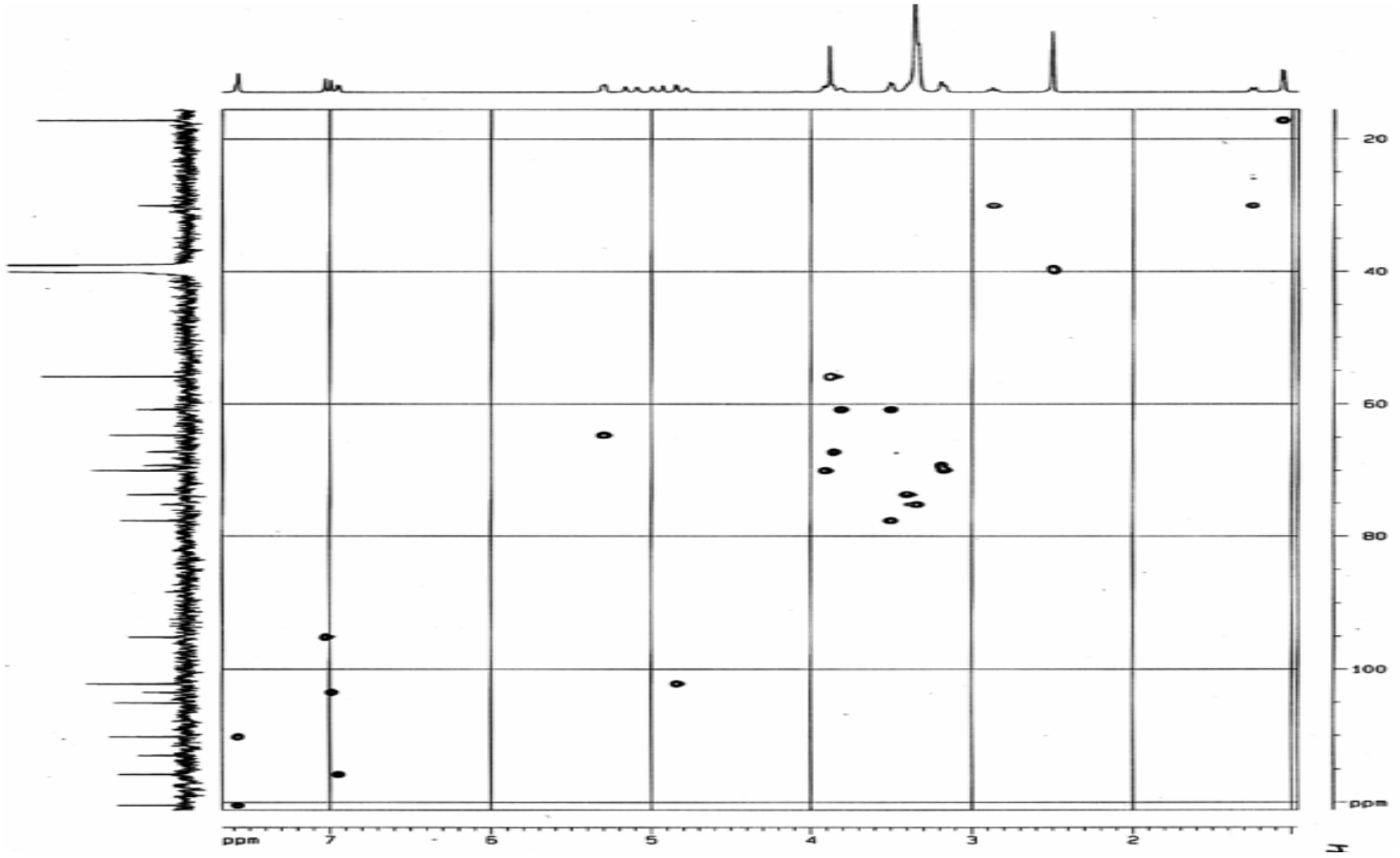


Figure 2. HMQC NMR spectrum of 2″-O-α-L-rhamnosyl-6-C-3″- deoxyglucosyl-3′-methoxyluteolin (compound I).

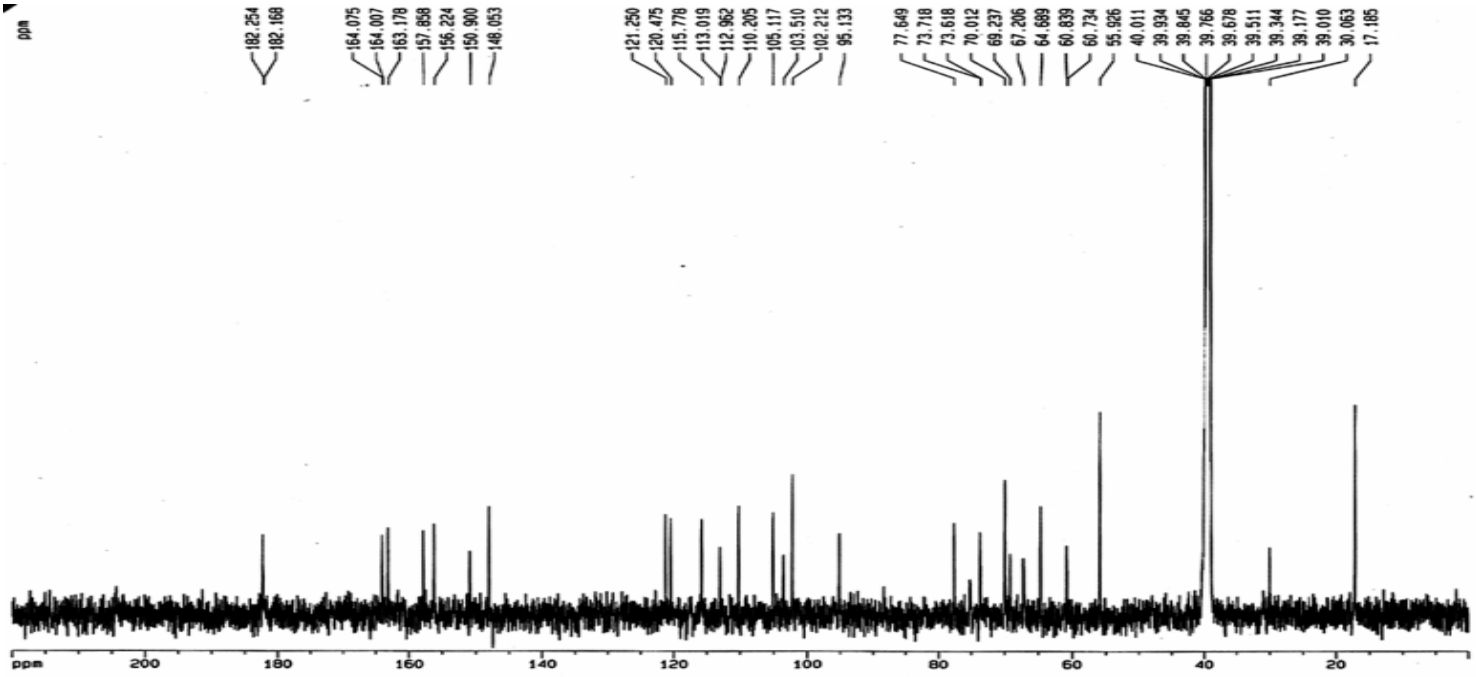


Figure 3. ^{13}C NMR spectrum of 2″-O-α-L-rhamnosyl-6-C-3″- deoxyglucosyl-3′-methoxyluteolin (compound I).

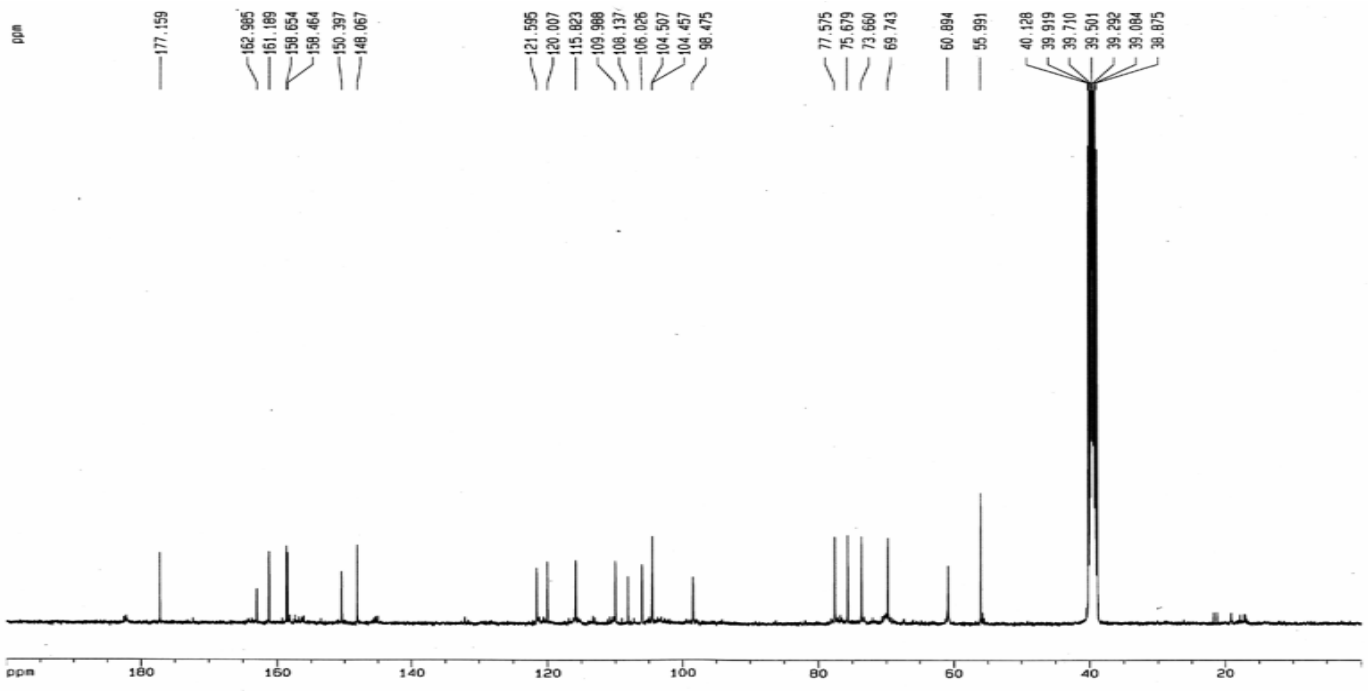


Figure 4. ^{13}C NMR spectrum of 6, 4′-dihydroxy-3′-methoxyflavone 7-O-glucoside (compound IV).

Table 1. ^{13}C NMR chemical shift assignments and DEPT of compound I and IV.

Carbon assignment	Compound I (DEPT)	Compound IV (DEPT)	Carbon assignment	Compound I (DEPT)	Compound IV (DEPT)
C-4	182.2(C)	177.2(C)	C-1‴	102.2(CH)	---
C-2	164.0(C)	163.0(C)	C-8	95.1(CH)	98.5(CH)
C-7	163.2(C)	161.2(C)	C-3″	30.1 (CH)	75.7(CH)
C-5	157.9(C)	106.0(CH)	C-2″	75.2 (CH)	73.7(CH)
C-9	156.2(C)	158.7(C)	C-5″	77.6(CH)	77.6(CH)
C-4′	150.9(C)	148.1(C)	C-4‴	73.7 (CH)	---
C-3′	148.1(C)	150.4(C)	C-1″	64.7(CH)	104.5(CH)
C-1′	121.3(C)	121.7(C)	C-2‴	69.9(CH)	---
C-6′	120.5(CH)	120.0(CH)	C-3‴	70.0(CH)	---
C-5′	115.8(CH)	115.8(CH)	C-5‴	69.2 (CH)	---
C-2′	110.0(CH)	110.0(CH)	C-4″	67.2(CH_2)	69.7(CH)
C-6	113.0(C)	158.5(C)	C-6‴	17.2(CH_3)	---
C-10	105.1(C)	108.0(C)	C-6″	60.8(CH_2)	60.9(CH_2)
C-3	103.5(CH)	104.5(CH)	OCH_3	55.9(CH_3)	56.0(CH_3)

different sources have been listed in Table 2. It is also suggest that the variant was statistically significant between different corn varieties. Total flavonoid levels were found to range from < 0.1 to >3.0% by dry weight; compound I, 0–0.187%; compound II, 0–0.149%; compound III, 0–0.134%; compound IV, 0–0.145%; compound V, 0–0.040%.

Conclusion

Five compounds including 2″-O-α-L-rhamnosyl-6-C-3″-deoxyglucosyl-3′- methoxyluteolin (compound I), ax-5″-methane-3′-methoxymaysin (compound II), ax-4″-OH-3′-methoxymaysin (compound III), *6, 4′-dihydroxy-3′-methoxyflavone-7-O-glucoside* (compound IV) and 7,4′-

Table 2. Flavonoids level of different corn silk lines (% dry weight).

Corn lines	Total flavonoids	Compound I	Compound II	Compound III	Compound IV	Compound V
CS1[a]	3.24	0.1875	0.1492	0.0134	0.0091 (a)	0.0403
YC	2.68	0.0802	0.0970	0.0275	0.0226	0.0063
HZ	1.83	0.1160	0.0538	ND	0.0079	ND
CS2	1.77 (a)[b]	0.1028	0.0426	ND	0.0447	ND
GZ	1.76 (a)	0.0537 (a)	0.1154	0.0072	0.0091 (a)	0.0170
LF	1.16	0.0634	0.0392	0.0086	0.0036 (b)	0.0018 (g)
ZJ033	0.99	0.0004 (l)	ND	0.0003	0.0010 (e)	0.0001
AKZ	0.92 (b)	0.0067 (h)	0.0372	0.0053 (c)	0.0120	0.0072
ZJ034	0.92 (b)	0.0155	0.0234	0.0108	0.0048	0.0026 (d)
MDJ	0.91 (b)	0.0542 (a)	0.0274	0.0080	0.0019	0.0030
TA108	0.88	0.0367	0.0198 (a)	0.0060 (a)	0.0031 (c)	0.0032
XA	0.82	0.0133 (e)	0.0199 (a)	0.0069	0.0036 (b)	0.0076
LA17	0.76	0.0027 (i)	0.0037	0.0005 (j)	0.0052	0.0018 (g)
BXJN	0.69	0.0296	0.0180 (bc)	0.0036 (d)	0.0011 (e)	0.0039 (c)
LY	0.66	0.0209 (c)	0.0136	0.0038	0.0011 (e)	0.0038 (c)
ZJ031	0.62 (c)	0.0256	0.0175 (c)	0.0063	0.0030 (c)	0.0055 (a)
JL	0.62 (c)	0.0200	0.0162 (d)	0.0046	0.0022 (d)	0.0052
ZJ032	0.62 (c)	0.0178 (d)	0.0147	0.0060 (a)	0.0027	0.0042 (b)
HEB	0.53	0.0175 (d)	0.0167 (d)	0.0056 (b)	0.0011 (e)	0.0043 (b)
WF	0.50 (d)	0.0220 (b)	0.0185 (b)	0.0052 (c)	0.0021 (d)	0.0026 (d)
BJ	0.50 (d)	0.0122 (f)	0.0154	0.0055 (d)	0.0025	0.0022 (e)
TY	0.45 (e)	0.0216 (bc)	0.0110	0.0026 (f)	0.0008 (f)	0.0018 (g)
YD2	0.45 (e)	0.0191	0.0208	0.0060 (a)	0.0013	0.0055 (a)
LS2	0.34 (f)	0.0043	0.0053 (f)	0.0028 (e)	0.0008 (f)	0.0008 (i)
TC	0.34 (f)	0.0085	0.0079 (e)	0.0018 (gh)	0.0004	0.0024
LA1	0.33 (f)	0.0074 (gh)	0.0088	0.0036 (d)	0.0007 (fg)	0.0006
XBP	0.33 (f)	0.0178 (d)	0.0078 (e)	0.0019 (g)	0.0006 (g)	0.0009 (hi)
ZJ	0.31	0.0126 (ef)	0.0098	0.0025 (f)	0.0007 (fg)	0.0010 (h)
HY1	0.29 (g)	0.0022 (ij)	0.0052 (f)	0.0017 (h)	ND	ND
GB303	0.29 (g)	0.0075 (g)	0.0067	0.0029 (e)	0.0007 (fg)	0.0012
TA981	0.25	0.0113	0.0129	0.0041	0.0006 (g)	0.0021 (ef)
WZ	0.23 (h)	ND[c]	ND	ND	ND	ND
ZZ	0.22 (h)	ND	ND	ND	ND	ND
NC	0.22 (h)	0.0018 (jk)	0.0021	0.0007 (i)	ND	ND
NT	0.18 (i)	0.0007 (l)	0.0011 (gh)	0.0006 (ij)	ND	ND
HD	0.17 (i)	0.0011 (kl)	0.0012 (g)	ND	ND	ND
DY	0.13 (j)	ND	0.0009 (gh)	0.0007 (i)	ND	0.0020 (f)
NY	0.12 (jk)	0.0008 (l)	0.0008 (gh)	ND	ND	ND
XS70	0.11 (k)	0.0007 (l)	0.0005 (h)	ND	ND	ND
WS	0.03	ND	ND	ND	ND	ND

a The abbreviations of letters and/or numbers in column 1 represent different corn sources
b Means (n = 3) with the same letter in the same column do not differ significantly ($P < 0.05$). Means without a letter are significantly different from all the other means in the same column.
c Not detectable.

dihydroxy-3′-methoxyflavone-2″-O-α-L-rhamnosyl-6-C fucoside (compound V) were successfully isolated and separated from corn silk. Among then compound I and IV were the first report from any natural source. Five classes of flavonoid contents of 40 corn silk lines collected from 16 provinces in China were reported, and corn variety was significant factor to influence the flavonoid content.

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