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TWO C-GLYCOSIDE FLAVONES FROM CORN (*ZEA MAYS*) SILK

Shuncheng Ren[1, 2*]

[1]National Engineering Laboratory of Wheat & Corn Further Processing, Henan University of Technology, Zhengzhou 450001, China. [2]School of Food Science and Technology, Henan University of Technology, Zhengzhou 450001, China

***Corresponding Author's E-mail:** scren@163.com

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Abstract

Background: Corn silk, a traditional Chinese herbal medicine in China, has been used to remedy nephritis, hepatitis, measles, hyperglycemia, tumor, etc. In this study, flavonoid monomers in corn silk were isolated and identified. This study could serve as a basis for determining the curative effects of these monomers on human diseases.

Method: Corn silk flavonoids were extracted using 80% (v/v) ethanol and isolated by repeated polyamide column and Toyopearl HW-40 column chromatography. Corn silk flavonoids were identified by UV spectroscopy, liquid chromatography/mass spectroscopy, and nuclear magnetic resonance spectroscopy.

Results: Two C-glycoside flavones, namely, *ax-4″-OH-3′-methoxymaysin* and a *3′-methoxymaysin* isomer, were isolated from corn silk and identified.

Conclusion: The two C-glycoside flavones were successfully isolated from corn silk and identified. This study is the first to report *3′-methoxymaysin* in corn silk.

Key Words: corn silk, C-glycoside flavone, monomer, isolation, identification.

Abbreviations: TLC - Thin-layer chromatography; ESI-MS - Electrospray ionization mass spectrometry; DEPT - Distortionless enhancement by polarization transfer.

Introduction

Corn silk refers to the silky tassels from the stigmas of corn, which belongs to the grass (*Gramineae*) family. Corn silk is a traditional herbal medicine in China. The earliest medical records of corn silk were found in 1476 in the "Southern Yunnan Materia Medica" of China. The material has been long used as a folk remedy in numerous countries worldwide, such as China, France, and Turkey. In China, corn silk has been used to remedy nephritis, hepatitis, measles, hyperglycemia, tumor, etc. (Ma and Gao, 1998). It exhibits diuretic, demulcent, and cholagogic effects (Namba, 1993; Wang and Guo, 1991), as well as anti-fatigue, anti-depression, and antioxidant activities (Hu et al., 2010; Mahmoudi and Ehteshami, 2010; Ebrahimzadeh et al., 2008; Bai et al., 2010). Native American Indians use corn silk to treat malaria, heart disease, and urinary infections (Hasanudin et al., 2012).

Corn silk contains numerous active ingredients, such as flavonoids, alkaloids, chlorogenic acid, and allantoin (Bushman, 2002). Flavonoids maysin, 3-methoxymaysine, and apigmaysin have been identified from corn silk (Waiss et al., 1979; Snook et al., 1995). In the current study, active constituent monomers in corn silk were isolated and identified. This study could serve as a basis for determining the curative effects of these monomers on human diseases.

Materials and Methods

General

UV–vis spectra were recorded using a UV-Vis2201 spectrophotometer. MS spectra were obtained with a Micromass ZSpray Mass Detector LC–MS spectrometer. NMR spectra were recorded on a Bruker Avance 500 MHz NMR spectrometer using standard pulse sequences. Chemical shifts were reported using δ values in ppm from tetramethylsilane. Thin-layer chromatography (TLC) was applied to analyze C-glycoside flavones on silica gel plates (Merck 60 F254) developed with chloroform/methanol/water/acetic acid mixtures (75:25:15:1, v/v) as the mobile phase. Chromatography was performed on a polyamide column (Zhejiang Taizhou Chemical Factory) and TOYOPEARL HW-40 (Tosoh Corporation).

Materials

Corn silk (Specimen No: YMX-2014-021) was provided by the Henan Academy of Agricultural Sciences and identified by the Laboratory of Identification and Resources of Chinese Medicine, Henan University of Traditional Chinese Medicine. Corn silk was crushed into a mean particle size of 0.4 mm for the extraction of C-glycoside flavones.

Extraction and isolation

A total mass of 2×4 kg of corn silk was pulverized. Then, 2 kg/batch of corn silk was added with 5 L of 80% (v/v) aqueous alcohol and extracted for three times in a container with a lid at 25±1 °C. The three extracts were combined together, filtered with five layers of gauze to remove the debris, condensed to 1.5 L (745 g/dry weight) at 45±1 °C by an evaporator (RE52CS, Shanghai Yarong Biochemical Instrument Factory, Shanghai, China), washed with 3×500 mL of petroleum ether, and then extracted using 3×500 mL of ethyl acetate. The ethyl acetate extract was collected together and concentrated to 160 mL (4.8 g/dry weight) at 45±1 °C by using a rotary evaporator.

The above concentrated solutions were dispersed in 100 mL of water. The resulting dispersion was fractioned using a polyamide column (80–100 mesh, 40×600 mm), eluted with water/ethanol (100:0–40:60, v/v), and then subjected to TLC performed on GF254 silica gel plates with chloroform/methanol/water/acetic acid mixtures (70:30:10:1, v/v) serving as the mobile phase. Five fractions marked as Fr. 1–Fr. 5 were collected (1203, 505, 145, 34, and 86 mg/dry weight, respectively). Re-chromatography of the five fractions was independently performed on a TOYOPEARL HW-40 chromatographic column (40×500 mm) with 15%–40% ethanol, respectively, which yielded flavonoids **I–V** (565, 232, 50, 19, and 30 mg/dry weight, respectively) with purity reaching 95% as analyzed by HPLC. The structure of the five flavonoids was identified by interpreting the spectra and referring to the related spectral data.

Results and Discussion

Five flavonoids **I–V** were isolated, and **II** and **III** were identified.

ax-5″-methane-3′-methoxymaysin **(II)**. This yellow-green powder is easily soluble in methanol. When fumigated with ammonia on a TLC plate, the powder became yellow. The UV spectrum of **II** showed two strong absorption bands at 272 (band I) and 351 nm (band II) in methanol. All of these characters suggest that **II** is a flavone derivative. Electrospray ionization mass spectrometry (ESI-MS) showed an ion peak at *m*/*z* 590 (M-H), and the molecular formula for **II** was determined to be $C_{28}H_{30}O_{14}$. The NMR spectra (^{1}H and ^{13}C) suggested the presence of a

luteolin and two simple sugar residue moieties (Table 1). Signals at δ 6.99 (1H, s) and δ 6.67 (1H, s) were assigned to H-3 in the C-ring and H-8 in the A-ring, respectively, and a singlet at δ 3.81 (3H, s) was attributed to an aromatic methoxyl group (OCH_3) at the 3′-position in the B-ring. Furthermore, ABX-type aromatic proton signals at δ 7.65 (2H, s) and δ 7.05 (1H, d, *J* = 7.1 Hz) were observed and assigned respectively to H-2′, H-6′, and H-5′ in the B-ring. The anomeric proton of the two sugar moieties appeared at δ 5.41 (1H, m) and δ 4.99 (1H, m) and assigned to H-1″ and H-1‴, respectively**.** The signals at δ 2.6–δ 4.8 were assigned to sugar residues on the basis of previously reported ^{1}H NMR spectra (Elliger et al., 1980). The signals at δ 71.3 and δ 99.3 in the ^{13}C NMR spectrum indicated a C-glycoside (Snook et al., 1995) and an O-glycoside (Toshihihiro et al., 2001), respectively. Two signals at δ 17.3 and δ 13.7 were assigned to two methyls (CH_3) through the ^{13}C NMR spectrum. The signal at δ 55.9 was assigned to one methoxy (OCH_3), and that at δ 99.3 was attributed to C-1‴ (Rhamnose). The C-signals of rhamnose were δ 99.3, δ 70.5, δ 70.3, δ 71.5, δ 69.0, and δ 17.4. The signal at δ 71.3 was attributed to C-1″ (4-ketofucosyl). The C-signals of 4-ketofucosyl were δ 71.3, δ 78.2, δ 78.3, δ 206.3, δ 75.6, and δ 19.1(Table 1).

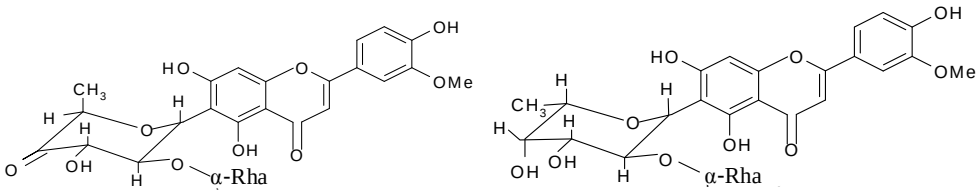
Table1: Assignments of ^{13}C NMR and DEPT data of flavonoid **II**

Carbon assignment	**II** δ (ppm) (DEPT)	Literature* δ (ppm)	Carbon assignment	**II** δ (ppm) (DEPT)	Literature* δ (ppm)
C-4″	206.3(C)	204.4	C-3	103.7(CH)	103.2
C-4	182.3(C)	181.6	C-1‴	99.3(CH)	100.1
C-2	163.7(C)	163.1	C-8	93.5(CH)	93.5
C-7	162.6(C)	163.1	C-3″	78.3(CH)	80.2
C-5	160.2(C)	160.5	C-2″	78.2(CH)	78.9
C-9	156.8(C)	156.4	C-5″	75.6(CH)	75.6
C-4′	148.2(C)	147.9	C-4‴	71.5(CH)	71.5
C-3′	151.9(C)	150.7	C-1″	71.3(CH)	71.2
C-1′	121.5(C)	121.3	C-2‴	70.5(CH)	70.4
C-6′	120.6(CH)	120.1	C-3‴	70.3(CH)	70.3
C-5′	116.0(CH)	115.7	C-5‴	69.0(CH)	68.2
C-2′	110.2(CH)	110.4	C-6‴	17.4(CH_3)	17.3
C-6	107.6(C)	107.6	C-6″	19.1(CH3)	13.7
C-10	103.3(C)	102.9	OCH_3	56.1(CH3)	55.9

* Snook et al., 1995.

As shown in Table 1, the assignments of the ^{13}C NMR and DEPT data of flavonoid **II** were consistent with the reported values in the literature (Snook et al., 1995), with C-6″ as the only exception. The δ value of **II** was 19.1 ppm, but the δ value in the literature was 13.1 ppm. This result was mainly due to C-6″ of **II** with an axial bond, whereas C-6″ in the literature involved an equatorial bond. Flavonoid **II** was the same isomer of the compound reported in the literature. When C-6″ was in an axial bond, the electron cloud density of C-6″ was reduced and the shielding effect was enhanced because the spatial position changed and was influenced by the electron withdrawing group (C=O) of C-4″, which caused the δ value to shift to the low field.

On the basis of the above analysis and comparison of reported spectral data (Table 1), flavonoid **II** was determined to be an isomer of *3′-methoxymaysin* (Fig. 1). ^{13}C NMR and DEPT spectral data are shown in Table 1.



II **III**

Figure 1: Structure of flavonoids **II** and **III**

ax-4″-OH-3′-methoxymaysin (**III**). This yellow-green powder is easily soluble in methanol. When fumigated with ammonia on a TLC plate, the powder became yellow. The UV spectrum of **III** showed two strong absorption bands at 272 (band I) and 351 nm (band II) in methanol. All of these characters suggest that **III** is a flavone derivative. ESI-MS yielded two ion peaks at *m*/*z* 594 (M+H) and 448 (M+H-rhamnose), and the molecular formula for **II** was determined to be $C_{28}H_{32}O_{14}$. The NMR spectra (^{1}H and ^{13}C) suggested the presence of a luteolin and two simple sugar residue moieties (Table 2). Signals at δ 6.57 (1H, s) and δ 6.85 (1H, s) were assigned to H-3 in the C-ring and H-8 in the A-ring, respectively, and a singlet at δ 3.89 (3H, s) was attributed to an aromatic methoxyl group (OCH_3) at the 3′-position in the B-ring. Furthermore, the ABX-type aromatic proton signals observed at δ 7.53 (2H, s) and δ 6.95 (1H, d, J = 6.2 Hz) in the B-ring were assigned to H-2′, H-6′, and H-5′. The anomeric protons of the two sugar moieties appearing at δ 5.03 (1H, m) and δ 4.75 (1H, m) were assigned to H-1″ and H-1‴, respectively. The signals at δ 2.6–δ 4.8 were assigned to sugar residues on the basis of previously reported ^{1}H NMR spectra (Elliger et al.,1980). A signal at δ 71.5 in the ^{13}C NMR spectrum indicated a C-glycoside (Snook et al., 1995). The signal at δ 100.7 in the ^{13}C NMR spectrum referred to a C-glycoside (Toshihihiro et al., 2001). Two signals at δ 17.8 and δ 17.1 were respectively assigned to two methyls (CH_3) through the ^{13}C NMR spectrum. The signal at δ 56.1 was assigned to one methoxy (OCH_3), and that at δ 100.7 was attributed to C-1‴ (Rhamnose). The C-signals of rhamnose were δ 100.7, δ 70.7, δ 70.6, δ 74.1, δ 68.3, and δ 17.8. The signal at δ 71.5 was attributed to C-1″ (fucosyl). The C-signals of fucosyl were δ 71.5, δ 73.9, δ 76.0, δ 74.1, δ 72.3, and δ 17.1 (Table 2).

Table 2: Assignments of ^{13}C NMR and DEPT data of flavonoid **III**

Carbon assignment	**III** δ (ppm) (DEPT)	Literature* δ (ppm)	Carbon assignment	**III** δ (ppm) (DEPT)	Literature* δ (ppm)	
C-4	181.9 (C)	181.7	C-1‴	100.7 (CH)	100.3	
C-2	163.6 (C)	163.3	C-8	94.1 (CH)	93.5	
C-7	163.1 (C)	162.4	C-4″	74.1 (CH)		
C-5	159.3 (C)	160.0	C-3″	76.0 (CH)	75.6	
C-9	156.8 (C)	156.4	C-2″	73.9 (CH)	73.3	
C-4′	148.2 (C)	147.9	C-5″	72.3 (CH)	72.0	
C-3′	150.9 (C)	150.7	C-4‴	71.6 (CH)	71.5	+C-4″
C-1′	121.6 (C)	121.4	C-1″	71.5 (CH)	71.3	
C-6′	120.5 (CH)	120.2	C-2‴	70.7 (CH)	70.4	
C-5′	116.0 (CH)	115.8	C-3‴	70.6 (CH)	70.3	
C-2′	110.2 (CH)	110.4	C-5‴	68.3 (CH)	67.9	
C-6	109.1 (C)	107.5	C-6‴	17.8 (CH_2)	17.3	
C-10	103.3 (C)	103.0	C-6″	17.1 (CH_2)	16.6	
C-3	103.7 (CH)	103.6	OCH_3	56.1 (CH_3)	56.0	

* Snook et al., 1995.

As shown in Table 2, the ^{13}C NMR data of flavonoid **III** were completely consistent with that reported in the literature (Snook et al., 1995). On the basis of the above analysis and comparison of reported spectral data (Table 2), compound **III** was determined to be *ax-4″-OH-3′-methoxymaysin* (Figure 1). ^{13}C NMR and DEPT spectral data are shown in Table 2.

Conclusion

Two C-glycoside flavones, including an isomer of *3′-methoxymaysin* (**II)** and *ax-4″-OH-3′-methoxymaysin* (**III**), were successfully isolated from corn silk and identified. Flavone **II** was reported for the first time in corn silk.

Conflict of Interest: The authors declare that there is no conflict of interest associated with this work.

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