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NEW FLAVONOID-*C*-GLYCOSIDES FROM *Triticum aestivum*

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*Two new flavonoid-C-glycosides named triticuside A (**1a**) and triticuside B (**1b**) were isolated from bran of* Triticum aestivum *L. The structures of the two new compounds were elucidated by spectral techniques including ^{1}H NMR, ^{13}C NMR as well as HSQC, HMBC, and COSY.*

Key words: *Triticum aestivum* L., triticuside A, triticuside B, flavonoid-C-glycoside, structure elucidation.

As a by-product of machining flour, wheat (*Triticum aestivum* L.) bran is a traditional Chinese medicine. Modern research has certified that wheat bran can relieve hematuria, diabetes, beriberi, and peripheral neuritis. However, its nutritional and chemical principles are not clear yet, especially for the healthy components such as flavonoids. Therefore, the primary objective of this study was to isolate and identify flavonoids in wheat bran by a phytochemical method. Previous phytochemical work on this plant had led to the isolation of about 26 flavonoids and most of them were C-glycosides [1-4]. Here, we describe the isolation and structure elucidation of two new flavonoid-C-glycosides from wheat bran.

Compounds **1a** and **1b** were obtained as a yellowish amorphous powder. Their molecular formula $C_{37}H_{38}O_{18}$ was determined based on their ESI-MS (771 $[M+H]^+$) and confirmed by ^{1}H NMR and ^{13}C NMR data. The ^{1}H NMR and ^{13}C NMR of compounds **1a** and **1b** were typical for the presence of a mixture of two structurally closely related compounds in the ratio of about 5:3. Detailed analysis of their ^{1}H NMR, ^{13}C NMR, COSY, HSQC, and HMBC spectra indicated the presence of flavonoid C-glycosides and sinapoyl structural units (see Table 1).

In the aromatic proton region of the ^{1}H NMR spectrum, four doublets were identified at δ 8.38, 7.02 and 8.02, 6.92 ppm (2H, d, J = 7 Hz), which showed the presence of two benzene moieties with the 1,4-position substituted, and two pairs of doublets at δ 7.30, 6.27, and 7.11, 5.97 ppm (1H, d, J = 16 Hz) were identified as the signals of two trans-double bonds in the sinapoyl structure unit. Acid hydrolysis of **1a** and **1b** together with four doublets at δ 4.51 ppm (d, J = 9 Hz), δ 4.66 ppm (d, J = 9 Hz), δ 5.00 ppm (d, J = 9.5 Hz), and δ 5.29 ppm (d, J = 9.5 Hz) indicated the presence of two galactosyl units and two arabinosyl units.

In the ^{13}C NMR spectrum, the signals at δ 182.5 ppm can be easily assigned to C-4. There were also two carbonyl signals at δ 166.0 and 164.9 ppm together with two pairs of *trans*-double bond carbons and four methyloxyl carbons which showed the presence of two sinapoyl structure units. We can also see the following structural segments: 12 signals of two galactosides, 10 signals of arabinosides, and 30 signals of the flavonoid core.

By analysis of the HMBC, the glucosyl unit can be ascribed to C-6 and C-8 not only from the downfield shift of the C-6 and C-8 signals but also from the observed correlation between the anomeric proton signals and the C-6 and C-8 carbons.

The positions of sinapoyl units could be determined from the ESI-MS, the HMBC spectrum, and the H-H COSY spectrum. In the ESI(+)-MS, a fragment at *m/z* 339 $[ara+sinapoyl]^+$ was seen and the fragment of $[gal+sinapoyl]^+$ could not been found, so the sinapoyl units should be attached to the arabinose units. The HMBC correlations between the protons (δ 5.88, 5.56 ppm) of the two arabinose units and the carbonyl carbons (δ 166.0, 164.9 ppm) of the two sinapoyl groups as well as the H-H COSY correlations between protons (δ 5.88, 5.56 ppm) and the anomeric protons (δ 5.00, 5.29 ppm) of the two arabinose units revealed that the sinapoyl groups was linked to the C-2 oxygen atoms of the two arabinose units.

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TABLE 1. ^{1}H NMR, ^{13}C NMR and HMBC Spectral Data for **1**a and **1b** in DMSO, δ, ppm

C atom	Compound **1a**			C atom	Compound **1b**		
	δ_C	δ_H	HMBC		δ_C	δ_H	HMBC
2	164.0 (q)		H-3	2	163.1 (q)		H-3
3	102.3 (t)	6.86 (s)		3	102.1 (t)	6.71 (s)	
4	182.5 (q)		H-3	4	182.5 (q)		H-3
5	158.1 (q)			5	158.0 (q)		
6	109.1 (q)		H-G_1	6	108.1 (q)		H-A_1
7	161.4 (q)		H-G_1, A_1	7	161.1 (q)		H-G_1, A_1
8	103.1 (q)		H-A_1	8	103.1 (q)		H-G_1
9	156.1 (q)		H-A_1	9	156.1 (q)		H-G_1
10	103.1 (q)		H-G_1	10	104.1 (q)		H-A_1
1′	121.2 (q)		H-2′,6′	1′	121.2(q)		H-2′,6′
2′	129.9 (t)	8.38 (d, 7 Hz)		2′	128.7(t)	8.02 (d, 7 Hz)	
3′	116.0 (t)	7.02 (d, 7 Hz)		3′	116.2 (t)	6.92 (d, 7 Hz)	
4′	162.0 (q)		H-3′,5′	4′	161.4 (q)		H-3′,5′
5′	116.2 (t)	7.02 (d, 7 Hz)		5′	116.0 (t)	6.92 (d, 7 Hz)	
6′	129.9 (t)	8.38 (d, 7 Hz)		6′	128.7 (t)	8.02 (d, 7 Hz)	
1″	124.6 (q)		H-8″	1″	124.1 (q)		H-8″
2″	106.1 (t)	6.90(s)	H-7″	2″	105.4 (t)	6.40 (s)	H-7″
			H-2″,6″,7″,	3″	147.8 (q)		H-2″,6″,6,7″ OMe
3″	147.9 (q)		OMe	4″	138.5 (q)		H-2″,6″
			H-2″,6″				H-2″,6″,7″,
4″	138.5 (q)		H-2″,6″,7″	5″	147.9 (q)		OMe
			OMe				H-7″
5″	147.9 (q)		H-7″	6″	105.4 (t)	6.40 (s)	H-2″,6″
			H-2″,6″	7″	144.5 (t)	7.11 (d, 16 Hz)	
6″	106.1 (t)	6.90(s)		8″	114.5 (t)	5.97 (d, 16 Hz)	H-A_2,7″,8″
7″	145.0 (t)	7.30 (d, 16 Hz)		9″	164.9 (q)		
8″	115.0 (t)	6.27 (d, 16 Hz)		OMe	56.0 (p)	3.69, 3.52	H-A_2
9″	166.0 (q)		H-A_2,7″,8″	A-1	72.0 (t)	5.29 (d, 9.5 Hz)	H-A_1, A_3
OMe	56.0 (p)	3.69, 3.52		2	71.5 (t)	5.56 (br t, 9.5 Hz)	
A-1	71.5 (t)	5.00 (d, 9.5 Hz)	H-A_2	3	72.0 (t)	3.41	
2	70.1 (t)	5.88 (br t, 9.5 Hz)	H-A_1,A_3	4	72.4 (t)	3.86	
3	72.0 (t)	3.41		5	70.1 (s)	3.06	H-G_2
4	72.4 (t)	3.86		G-1	73.9 (t)	4.51 (d, 9 Hz)	H-G_1, G_3
5	70.5 (s)	3.06		2	70.7 (t)	3.80	
G-1	74.1 (t)	4.66 (d, 9 Hz)	H-G_2	3	73.9 (t)	3.41	
2	70.7 (t)	3.80	H-G_1, G_3	4	68.3 (t)	3.90	
3	73.9 (t)	3.41		5	80.0 (t)	3.91	
4	68.3 (t)	3.90		6e	59.9 (s)	3.60	
5	80.8 (t)	3.60					
6	61.2 (s)	3.60					

(^{13}C NMR 125 MHz, ^{1}H NMR 500 MHz, all chemical shifts as internal TMS, carbon multiplicities determined by DEPT (q (C), t (CH), s (CH_2), p (CH_3), A - arabinosyl, G - galactosyl).

On the basis of the above evidence, the structure of compound **1a** can be elucidated as apigenin-6-C-galactoside-8-C-(2-sinapoyl) arabinoside, named triticuside A. The structure of compound **1b** can be elucidated as apigenin-6-C-(2-sinapoyl) arabinoside-8-C-galactoside, named triticuside B.

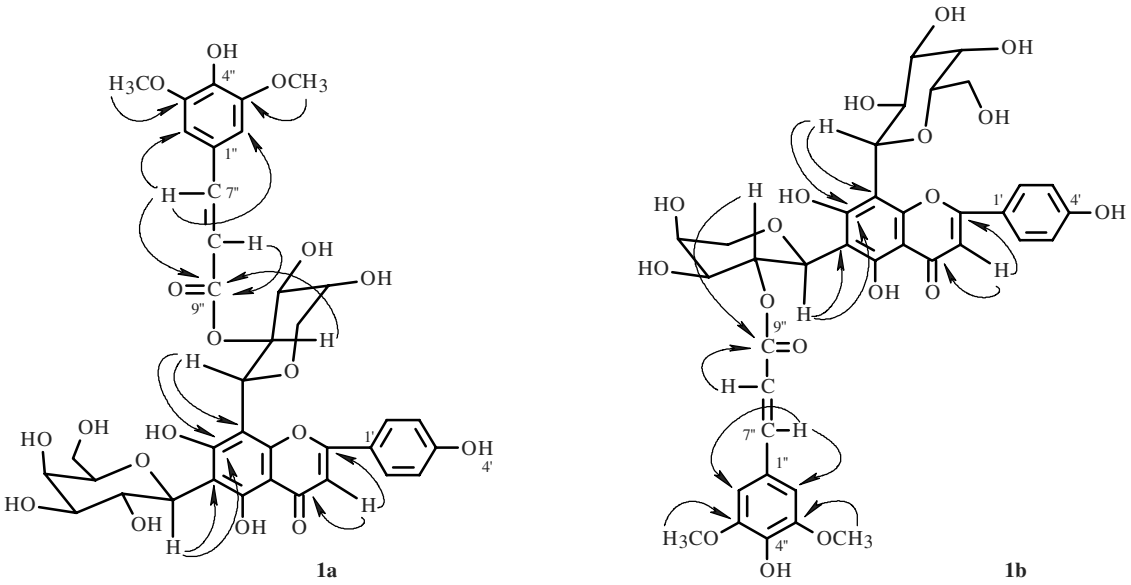

Fig. 1. The key HMBC correlations of compounds **1a** and **1b**.

EXPERIMENTAL

General Methods. ^{1}H NMR and ^{13}C NMR, DEPT, COSY, HMQC, and HMBC spectra: Bruker AM-500 spectrometers operating at 500 MHz; ESI-MS: Agilent 1100 LC/MSD SL.

Plant Material. Wheat (*Triticum aestivum* L.) variety Ningmai 9 was harvested in 2002 in the Jiangpu Experiment Station of Nanjing Agricultural University. A voucher specimen was deposited in Nanjing Botanical Garden Mem. Sun Yat-Sen, Nanjing, Jiangsu, China. Bran was obtained via a Branbendar Junior miller.

Extraction and Purification. The wheat bran (5.0 kg) was extracted with ethanol at room temperature. After removal of ethanol, the water suspension was re-extracted with petroleum ether and *n*-BuOH. The obtained *n*-BuOH portion (200 g) was subjected to D101 macroporous resin (H_2O>EtOH) to furnish six fractions (Fr. 1~6). Fraction 2 was chromatographed on an RP-C_{18} column (H_2O>MeOH) and then Sephadex LH-20 to afford **1a** and **1b** (9.2 mg).

Acid Hydrolysis of Compound 1a+1b. The sample was placed on the HPTLC plate and bathed with HCl for 4 hours and then chromatographed with BAW (*n*-BuOH : EtOH : H_2O 4:1:5). The same points can be seen as galactosyl and arabinosyl.

Triticuside A + Triticuside B (1a+1b). Yellow amorphous powder (MeOH-H_2O); mp 293-297°C; The UV (λ_{max}, nm): 337 (I band), 274 (II band) (MeOH); 377 (I band), 275 (II band) (MeOH+MeONa); 343, 380 (I band), 275 (II band) (MeOH+$AlCl_3$); 338 (I band), 283 (II band) (MeOH+NaOAc) and IR (KBr, ν_{max}, cm^{-1}): 3300, 2935, 1690, 1600, 1560, 1490, 1235, 1162, 1050, 820; ESI-MS, *m/z*: 771 $[M+H]^+$ spectra indicate that the molecular weight is 770; combined with the data of ^{1}H NMR and ^{13}C NMR, the molecular formula can be deduced to be $C_{37}H_{38}O_{18}$. For ^{1}H NMR and ^{13}C NMR, as well as HMBC spectral data see Table 1.

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