

Flavonoids from Tartary Buckwheat Seeds

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Nine flavonoids were isolated from the seeds of tartary buckwheat. Their structures were elucidated as quercetin (**1**), rutin (**2**), vitexin (**3**), orientin (**4**), hyperin (**5**), tricin-7-O- β -D-glucopyranoside (**6**), chrysoeriol (**7**), tectochrysin (**8**) and luteolin-7-O- β -D-glucopyranoside (**9**), by means of spectroscopic methods, especially by ¹H and ¹³C NMR, FAB-MS and GC, as well as by chemical methods and comparison with literature data.

Key Words: Tartary buckwheat seed, Flavonoid.

INTRODUCTION

Flavonoids are a group of naturally occurring plant compounds that serve many functions *e.g.*, antioxidant, anti-tumor, antiinflammatory, antituberculosis, antimalarial, antimicrobial and antiviral, *etc.*^{1,2}. The seeds of tartary buckwheat (*Fagopyrum tataricum* Gaertn.) serve as a rich source of flavonoids and have been popularly recognized as an excellent selective antioxidant and hypolipidaemic nutrient food in China and many other countries of the world³. In order to make full use of tartary buckwheat, our recent research led to the isolation of 9 flavonoids from the seeds of tartary buckwheat.

EXPERIMENTAL

Acid hydrolysis of compounds and determination of the absolute configuration of the monosaccharide: A solution of compound (5.0 mg, **2-6** and **9**) in 2 mol/L HCl-dioxane (1:1, 1 mL) was refluxed in a water bath at 90 °C for 2 h. After removal of dioxane, the solution was extracted with EtOAc (1 mL $\times$ 3). The aqueous layer was neutralized by passing through an Amberlite MB-3 resin column eluted with H₂O, then concentrated and dried to furnish a monosaccharide residue. Then, the sugars were detected by TLC analysis [CHCl₃-CH₃OH-H₂O-HOAc (15:6:2:3), detection solution: aniline-phthalic acid] against the standard samples. The residue was dissolved in pyridine (0.2 mL) and then a pyridine solution (0.3 mL) of L-cysteine methyl ester hydrochloride (5 mg) was added to the solution. The mixture was kept at 60 °C for 1.5 h, dried *in vacuo* and trimethylsilylated with hexamethyl-

disilazane-trimethylchlorosilane (HMDS-TMCS) (0.1 mL) at 60 °C for 1 h. After being partitioned between *n*-hexane (0.5 mL) and H₂O (0.5 mL), the *n*-hexane extract was concentrated and analyzed by GC under the following conditions: HP-5 MS fused silica capillary column (30 m $\times$ 0.25 mm, film thickness 0.25 μ m), column temperature at 230 °C, injection temperature at 250 °C, N₂ as carrier gas. The sugars were confirmed by comparison of the retention times of their derivatives with standard samples [retention time, D-glucose (21.7 min), D-galactose (25.4 min) and L-rhamnose (19.1 min)]. The presence of L-rhamnose in **2**, D-galactose in **5**, D-glucose in **2**, **3**, **4**, **6** and **9** were detected.

RESULTS AND DISCUSSION

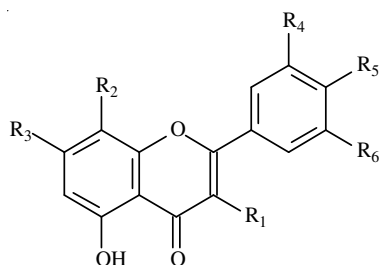
The seeds of tartary buckwheat were collected in September 2010 from Ring County of China and extracted three times with 95 % aqueous EtOH at room temperature. The extract was dissolved in 50 % aqueous EtOH and extracted with petroleum ether and EtOAc. The EtOAc extract was separated by repeated column chromatography (Silica-gel, Diaion HP-20 and Sephadex LH-20) and recrystallization to give nine flavonoid compounds. On the basis of chemical (acid hydrolysis) and spectral analyses (UV, IR, MS and NMR) and comparison with the literature data, their structures were elucidated as quercetin (**1**)^{4,5}, rutin (**2**)^{6,7}, vitexin (**3**)⁸, orientin (**4**)⁹, hyperin (**5**)^{10,11}, tricin-7-O- β -D-glucopyranoside (**6**)¹², chrysoeriol (**7**)^{13,14}, tectochrysin (**8**)¹⁵ and luteolin-7-O- β -D-glucopyranoside (**9**)^{16,17}. Although quercetin, rutin, vitexin, orientin and hyperin have been previously isolated from tartary buckwheat¹⁸, this is first report on the occurrence of tricin-7-O-glucopyranoside,

TABLE-1
¹H-NMR DATA OF COMPOUNDS 1-9 (δ_H in DMSO-*d*₆, 400 MHz, *J*: Hz)

Proton	1	2	3	4	5	6	7	8	9
3			6.76 (s)	6.64 (s)		7.01 (s)	6.88 (s)	6.68 (s)	6.74 (s)
6	6.18 (d, 2.0)	6.21 (d, 2.0)	6.28 (s)	6.25 (s)	6.19 (d, 2.4)	6.92 (d, 1.9)	6.22 (d, 2.0)	6.39 (d, 2.1)	6.43 (d, 1.9)
8	6.38 (d, 2.0)	6.40 (d, 2.0)			6.41 (d, 2.4)	6.43 (d, 1.9)	6.49 (d, 2.0)	6.51 (d, 2.1)	6.79 (d, 1.9)
2'	7.62 (d, 2.0)	7.51 (d, 2.1)	7.99 (d, 8.1)	7.48 (d, 2.0)	7.51 (d, 1.7)	7.32 (s)	7.54 (m)	7.85 (m)	7.41 (d, 2.1)
3'			6.88 (d, 8.1)					7.48 (m)	
4'								7.54 (m)	
5'	6.88 (d, 8.1)	6.82 (d, 8.2)	6.88 (d, 8.1)	6.85 (d, 8.4)	6.81 (d, 8.4)		6.98 (d, 8.4)	7.48 (m)	6.88 (d, 8.0)
6'	7.56 (dd, 8.1, 2.0)	7.60 (dd, 8.2, 2.1)	7.99 (d, 8.1)	7.52 (dd, 8.4, 2.0)	7.66 (dd, 8.4, 1.7)	7.32 (s)	7.54 (dd, 8.4, 2.0)	7.85 (m)	7.46 (dd, 8.0, 2.1)
C ₅ -OH	12.48 (s)	12.58 (s)	13.21 (s)	13.16 (s)	12.62 (s)		12.95 (s)	12.71 (s)	12.98 (s)
OCH ₃						3.85 (s)	3.90 (s)	3.89 (s)	
Anomeric H									
Glu		5.41 (d, 7.9)	4.68 (d, 7.6)	4.67 (d, 7.8)		5.02 (d, 7.4)			5.08 (d, 7.5)
Rha		4.38 (br s)							
Gal					5.36 (d, 7.8)				

TABLE-2
¹³C-NMR (DEPT) DATA OF COMPOUNDS 1-9 (δ_C in DMSO-*d*₆, 100 MHz)

Carbon	1	2	3	4	5	6	7	8	9
2	146.8 (C)	156.6 (C)	163.9 (C)	164.1 (C)	156.2 (C)	164.5 (C)	163.8 (C)	163.8 (C)	164.3 (C)
3	135.6 (C)	133.4 (C)	102.4 (CH)	102.3 (CH)	133.8 (C)	103.9 (CH)	103.8 (CH)	104.3 (CH)	103.4 (CH)
4	175.6 (C)	177.2 (C)	182.1 (C)	182.0 (C)	177.6 (C)	182.1 (C)	181.9 (C)	182.2 (C)	181.9 (C)
5	160.1 (C)	161.2 (C)	161.1 (C)	160.5 (C)	161.0 (C)	157.3 (C)	157.4 (C)	162.0 (C)	159.8 (C)
6	99.0 (CH)	98.7 (CH)	98.2 (CH)	98.1 (CH)	98.9 (CH)	95.4 (CH)	98.8 (CH)	98.1 (CH)	99.5 (CH)
7	163.7 (C)	164.1 (C)	162.6 (C)	162.6 (C)	164.3 (C)	163.2 (C)	164.2 (C)	165.2 (C)	164.3 (C)
8	93.2 (CH)	93.6 (CH)	104.6 (C)	104.6 (C)	93.7 (CH)	99.5 (CH)	94.6 (CH)	92.6 (CH)	94.5 (CH)
9	156.0 (C)	156.5 (C)	156.2 (C)	156.0 (C)	156.9 (C)	161.2 (C)	161.7 (C)	157.4 (C)	157.3 (C)
10	102.5 (C)	104.1 (C)	104.2 (C)	104.1 (C)	104.0 (C)	105.4 (C)	103.3 (C)	105.6 (C)	105.2 (C)
1'	121.8 (C)	121.3 (C)	121.6 (C)	122.0 (C)	121.3 (C)	120.5 (C)	120.4 (C)	131.5 (C)	121.0 (C)
2'	114.9 (CH)	115.7 (CH)	129.1 (CH)	114.0 (CH)	115.4 (CH)	104.6 (CH)	110.2 (CH)	126.2 (CH)	113.7 (CH)
3'	144.8 (C)	144.8 (C)	115.9 (CH)	145.8 (C)	145.0 (C)	148.3 (C)	150.8 (C)	129.0 (CH)	145.6 (C)
4'	147.7 (C)	148.4 (C)	160.4 (C)	149.7 (C)	148.6 (C)	140.1 (C)	147.9 (C)	131.4 (CH)	149.9 (C)
5'	115.4 (CH)	121.7 (CH)	115.9 (CH)	115.7 (CH)	116.1 (CH)	148.3 (C)	115.8 (CH)	129.0 (CH)	115.8 (CH)
6'	119.6 (CH)	116.5 (CH)	129.1 (CH)	119.4 (CH)	122.4 (CH)	104.6 (CH)	121.8 (CH)	126.2 (CH)	119.3 (CH)
CH ₃ O						55.6 (CH ₃)	56.1 (CH ₃)	55.7 (CH ₃)	
Sugar	Glu	Rha	Glu	Glu	Gal	Glu			Glu
1	101.2 (CH)	100.8 (CH)	73.8 (CH)	73.4 (CH)	101.9 (CH)	100.8 (CH)			100.1 (CH)
2	74.3 (CH)	70.1 (CH)	70.8 (CH)	70.8 (CH)	71.3 (CH)	73.3 (CH)			73.1 (CH)
3	76.3 (CH)	70.6 (CH)	78.6 (CH)	78.8 (CH)	73.4 (CH)	77.4 (CH)			77.5 (CH)
4	70.1 (CH)	71.9 (CH)	70.5 (CH)	70.7 (CH)	68.1 (CH)	69.8 (CH)			69.7 (CH)
5	75.8 (CH)	68.2 (CH)	81.7 (CH)	82.0 (CH)	75.8 (CH)	76.6 (CH)			76.4 (CH)
6	67.0 (CH ₂)	17.5 (CH ₃)	61.3 (CH ₂)	61.7 (CH ₂)	60.3 (CH ₂)	60.8 (CH ₂)			60.6 (CH ₂)



Flavonoid	R ₁	R ₂	R ₃	R ₄	R ₅	R ₆
1	OH	H	OH	OH	OH	H
2	OGlc-(6→1)-Rha	H	OH	OH	OH	H
3	H	Glc	OH	H	OH	H
4	H	Glc	OH	OH	OH	H
5	OGal	H	OH	OH	OH	H
6	H	H	OGlc	OCH ₃	OH	OCH ₃
7	H	H	OH	OCH ₃	OH	H
8	H	H	OCH ₃	H	H	H
9	H	H	OGlc	OH	OH	H

chrysoeriol, tectochrysin and luteolin-7-O-glucopyranoside in this genus.

Quercetin (1), yellow needles (CHCl₃-MeOH), m.p. 312-314 °C. UV spectrum (MeOH, λ_{max}, nm): 208 sh, 255, 284, 379 sh; IR spectrum (KBr, ν_{max}, cm⁻¹): 3406 (OH), 1665 (C=O), 1611, 1562, 1522, 1452 (Ar); negative FAB-MS *m/z*: 301 [M-H]⁻. ¹H and ¹³C NMR (Tables 1 and 2).

Rutin (2), yellow powder (CHCl₃-MeOH), m.p. 191-192 °C. UV spectrum (MeOH, λ_{max}, nm): 207, 259 sh, 261, 356 sh; IR spectrum (KBr, ν_{max}, cm⁻¹): 3422 (OH), 1656 (C=O), 1604, 1567, 1506, 1456 (Ar), 1363 (CH₃), 1064, 1025 (glycosidic bond); negative FAB-MS *m/z*: 609 [M-H]⁻. ¹H and ¹³C NMR (Tables 1 and 2).

Vitexin (3), yellow crystal (MeOH), m.p. 269-271 °C. UV (MeOH, λ_{max}, nm): 250, 267 sh, 337 sh; IR spectrum (KBr, ν_{max}, cm⁻¹): 3278 (OH), 1660 (C=O), 1608, 1500 (Ar); negative FAB-MS *m/z*: 431 [M-H]⁻. ¹H and ¹³C NMR (Tables 1 and 2).

Orientin (4), yellow crystal (MeOH), m.p. 261-263 °C. UV (MeOH, λ_{max} , nm): 248 sh, 258 sh, 324; IR spectrum (KBr, ν_{max} , cm^{-1}): 3312 (OH), 1658 (C=O), 1612, 1500 (Ar); negative FAB-MS m/z : 447 [M-H]⁻. ¹H and ¹³C NMR (Tables 1 and 2).

Hyperin (5), pale yellow needles (MeOH), m.p. 235-237 °C. UV spectrum (MeOH, λ_{max} , nm): 206 sh, 257, 299, 362 sh; IR spectrum (KBr, ν_{max} , cm^{-1}): 3423 (OH), 1661 (C=O), 1604, 1562, 1501, 1463 (Ar); negative FAB-MS m/z : 463 [M-H]⁻. ¹H and ¹³C NMR (Tables 1 and 2).

Tricin-7-O- β -D-glucopyranoside (6), yellow needles (MeOH), m.p. 186-188 °C. UV spectrum (MeOH, λ_{max} , nm): 250, 266 sh, 352 sh; IR spectrum (KBr, ν_{max} , cm^{-1}): 3447 (OH), 1650 (C=O), 1607, 1510, 1478 (Ar); negative FAB-MS m/z : 491 [M-H]⁻. ¹H and ¹³C NMR (Tables 1 and 2).

Chrysoeriol (7), yellow needles, m.p. 323-325 °C. UV spectrum (MeOH, λ_{max} , nm): 247 sh, 267, 345 sh; IR spectrum (KBr, ν_{max} , cm^{-1}): 3356 (OH), 1652 (C=O), 1626, 1597, 1508 (Ar); negative FAB-MS m/z : 299 [M-H]⁻. ¹H and ¹³C NMR (Tables 1 and 2).

Tectochrysin (8), pale yellow needles (EtOH), m.p. 162-165 °C. UV spectrum (MeOH, λ_{max} , nm): 248, 269 sh, 312 sh; IR spectrum (KBr, λ_{max} , cm^{-1}): 3441 (OH), 1620 (C=O), 1615, 1595 (Ar); negative FAB-MS m/z : 267 [M-H]⁻. ¹H and ¹³C NMR (Tables 1 and 2).

Luteolin-7-O- β -D-glucopyranoside (9), yellow crystal (MeOH), m.p. 252-254 °C. UV spectrum (MeOH, λ_{max} , nm): 255 sh, 267, 351 sh; IR spectrum (KBr, ν_{max} , cm^{-1}): 3375 (OH), 1665 (C=O), 1560, 1510 (Ar), 1095, 1030 (glycosidic bond); negative FAB-MS m/z : 447 [M-H]⁻. ¹H and ¹³C NMR (Tables 1 and 2).

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