

Structures of Three New Acylated Flavonol Glycosides from *Astragalus complanatus* R. BR.¹⁾

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Three new acylated flavonol glycosides (1—3) were isolated from Astragali Semen, seeds of *Astragalus complanatus* R. BR. (Leguminosae). The structures of 1, 2 and 3 were elucidated as 3-*O*- β -D-glucopyranosyl-4'-*O*-(3'''-*O*-dihydrophaseoyl- β -D-glucopyranosyl) rhamnocitrin, 3-*O*-[5'''-*O*-*p*-coumaroyl- β -D-apiofuranosyl(1''' \rightarrow 2'')- β -D-glucopyranosyl] rhamnocitrin and 3-*O*-[5'''-*O*-feruloyl- β -D-apiofuranosyl(1''' \rightarrow 2'')- β -D-glucopyranosyl] rhamnocitrin, respectively.

Keywords Astragali Semen; *Astragalus complanatus*; Leguminosae; dihydrophaseic acid; *p*-coumaric acid; ferulic acid; rhamnocitrin; acylated flavonol glycoside

In previous papers we reported the isolation of flavonoids,²⁾ six triterpene glycosides³⁾ and a novel acylated flavonoid glycoside called complanatin⁴⁾ from Astragali Semen, seeds of *Astragalus complanatus* R. BR. (Leguminosae). Our continuing study on this crude drug has revealed the occurrence of three other new flavonol glycosides (1—3) acylated with dihydrophaseic acid,⁵⁾ *p*-coumaric acid and ferulic acid, respectively. We describe here the isolation and characterization of these three new constituents.

The methanol extract of Astragali Semen (4.5 kg) was partitioned between *n*-hexane and 80% MeOH, and then the MeOH extract was further shaken with 1-BuOH and water. Removal of the solvent of the organic layer gave a residue which was subjected to normal and reversed phase column chromatographies to yield three acylated flavonol glycosides **1** (31 mg), **2** (64 mg) and **3** (52 mg).

Compound **1**, a pale yellow powder, $[\alpha]_D^{25} -33.8^\circ$ (MeOH), showed a peak due to $[M+H]^+$ at m/z 889 in the positive fast atom bombardment mass spectrum (FAB-MS), and absorptions at $\lambda_{\max}^{\text{MeOH}}$ (nm) 267 (log ϵ , 4.59) and 345 (log ϵ , 4.42) in the ultraviolet (UV) spectrum. The infrared (IR) spectrum exhibited absorption bands due to an α,β -unsaturated carboxylic ester (1660 cm^{-1}) and an aromatic ring (1602 cm^{-1}) together with hydroxyl groups (3432 cm^{-1}). The proton nuclear magnetic resonance (¹H-NMR) spectrum (in dimethyl sulfoxide (DMSO)-*d*₆) of **1** displayed signals due to a 3,5,7,4'-tetrahydroxyflavonol derivative at δ 12.56 (1H, s, 5-OH), 8.17 (2H, d, $J=8.8$ Hz, 2',6'-H), 7.20 (2H, d, $J=8.8$ Hz, 3',5'-H), 6.77 (1H, d, $J=2.2$ Hz, 8-H), 6.40 (1H, d, $J=2.2$ Hz, 6-H). In addition, one methyl group at δ 3.87 (3H, s) was shown to connect to C-7-OH by observation of the nuclear Overhauser effect (NOE) between the methoxy group and 6-H, 8-H. Therefore, the flavonol part in **1** was characterized as rhamnocitrin. Moreover, the carbon-13 nuclear magnetic resonance (¹³C-NMR) spectrum of **1** as listed in Table I revealed that each mole of β -D-glucosyl moiety connected to both C-3-OH and C-4'-OH of rhamnocitrin by comparing chemical shifts with those of rhamnocitrin 3-*O*-glycoside^{2,6)} [C-3, δ 134.0 (+0.7); C-1', δ 123.8 (+3.1); C-3', 5', δ 115.9 (+0.8); C-4', δ 159.1 (-1.0)]. The remaining ¹H-NMR signals were assignable to dihydrophaseic acid⁵⁾ as follows: δ 0.83 (3H, s, 1-Me), 1.02 (3H, s, 5-Me), 1.55 (1H, dd, $J=13.2, 10.3$ Hz, 2-H_a), 1.63 (1H, dd, $J=13.2, 10.3$ Hz, 4-H_a), 1.72 (1H, dd, $J=13.2, 6.6$ Hz, 2-H_b), 1.88 (1H, dd, $J=13.2, 6.6$ Hz, 4-H_b), 2.08 (3H, s, 3'-Me), 3.56 (1H, d, $J=7.3$ Hz, 7-H_a), 3.75 (1H, d, $J=7.3$ Hz, 7-H_b), 3.92 (1H,

m, 3-H), 5.82 (1H, s, 4'-H), 6.50 (1H, d, $J=16.1$ Hz, 2'-H), 7.97 (1H, d, $J=16.1$ Hz, 1'-H), which corresponded to a NaBH₄-reductive product of phaseic acid. In addition, the ¹³C-NMR spectrum (Table I) also suggested the occurrence of dihydrophaseic acid moiety. On the other hand, enzymatic hydrolysis of **1** afforded a deglycosyl product **1a** which showed a peak at m/z 727 due to $[M+H]^+$ in the positive FAB-MS spectrum. The ¹³C-NMR signals (Table I) of **1a** indicated that the C-3-OH was free and that the acylated glucosyl moiety was linked to C-4'-OH, and acylation position was at the C-3'''-OH of 4'-*O*-glucosyl moiety by the acylation shifts^{4,7)} [C-2''', δ 71.3 (-1.9); C-3''', δ 76.9 (+0.5); C-4''', δ 67.6 (-2.0) in glucosyl moiety]; this was also confirmed by the ¹H-NMR (in pyridine-*d*₅) observation of signals at δ 4.46 (1H, dd, $J=8.1, 9.2$ Hz, glc 2-H), 6.07 (1H, dd, $J=9.2, 9.5$ Hz, glc 3-H), 4.55 (1H, dd, $J=9.2, 9.5$ Hz, glc 4-H) and ¹H-¹H correlation spectroscopy (COSY). The full structure of **1** was thus characterized as 3-*O*- β -D-glucopyranosyl-4'-*O*-(3'''-*O*-dihydrophaseoyl- β -D-glucopyranosyl) rhamnocitrin.

Compound **2**, a pale yellow powder, $[\alpha]_D^{25} -148.4^\circ$ (MeOH), showed peaks due to $[M+Na]^+$ at m/z 763 and $[aglycone+H]^+$ at m/z 301 in the positive FAB-MS spectrum, UV absorptions at $\lambda_{\max}^{\text{MeOH}}$ (nm) 267 (log ϵ 4.58), 317 (log ϵ 4.12) and IR absorptions (cm^{-1}) at 3444 (OH), 1660 (conjugated carbonyl) and 1606 (aromatic ring). The ¹H- and ¹³C-NMR spectrum (in DMSO-*d*₆) (Table I) suggested that **2** was a rhamnocitrin 3-*O*-glycoside, which was supported by the NOE experiment between 7-OMe and 6, 8-H. Meanwhile, the ¹H-NMR (in DMSO-*d*₆) signals due to aromatic and olefinic protons at δ 9.62 (1H, s), 7.31 (2H, d, $J=8.8$ Hz), 7.19 (1H, d, $J=15.8$ Hz), 6.76 (2H, d, $J=8.8$ Hz), 6.06 (1H, d, $J=16.1$ Hz) could be assigned to a *p*-coumaroyl residue. Therefore, **2** was estimated as a rhamnocitrin 3-*O*-glycoside acylated with *p*-coumaric acid, which was also apparent from the evidence of the ¹³C-NMR spectrum.⁸⁾ Compound **2**, on saponification, provided a product **2a**, a pale yellow solid, $[\alpha]_D^{25} -39.7^\circ$ (MeOH). The peak at m/z 595 resulting from $[M+H]^+$ in the positive FAB-MS spectrum and the ¹H-NMR signals (in DMSO-*d*₆) at δ 12.67 (1H, s, 5-OH), 10.26 (1H, s, 4'-OH), 8.16 (2H, d, $J=8.8$ Hz, 2', 6'-H), 6.93 (2H, d, $J=8.8$ Hz, 3', 5'-H), 6.67 (1H, d, $J=2.2$ Hz, 8-H), 6.33 (1H, d, $J=2.2$ Hz, 6-H), 5.72 (1H, d, $J=7.3$ Hz, glc 1''-H), 5.45 (1H, s, api 1'''-H) and 3.84 (3H, s, 7-OMe) suggested that **2a** was a rhamnocitrin 3-*O*-glycoside whose sugar part was assignable to β -D-apiofuranosyl(1 \rightarrow 2)- β -D-gluco-

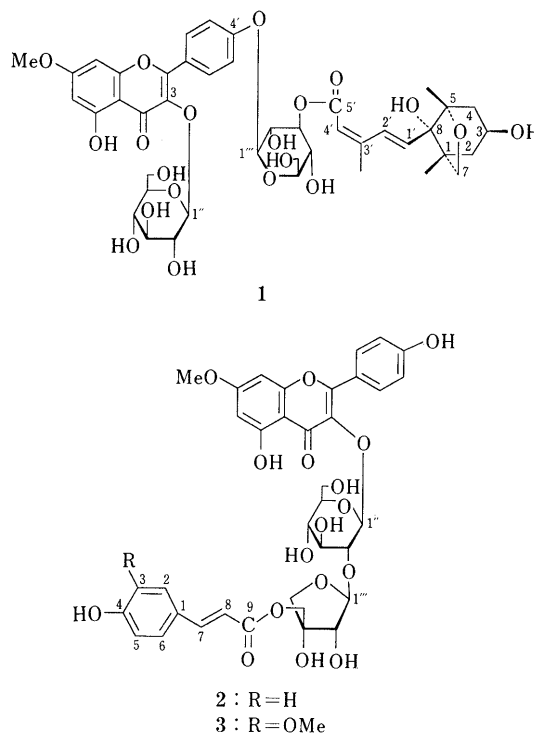
TABLE I. ^{13}C -NMR Spectral Data for **1**, **1a**, **2**, **2a** and **3** (in $\text{DMSO}-d_6$)

	1	1a	2	2a	3
Flavonol moiety			Flavonol moiety		
C-2	156.4	146.4	C-2	155.9	155.9
C-3	134.0	136.6	C-3	133.1	133.4
C-4	177.7	176.2	C-4	177.4	177.4
C-5	161.0	160.4	C-5	161.0	161.1
C-6	98.0	97.5	C-6	97.8	97.9
C-7	165.3	165.1	C-7	164.9	165.2
C-8	92.4	92.1	C-8	92.0	92.2
C-9	155.9	156.2	C-9	156.1	156.3
C-10	105.2	104.1	C-10	105.1	105.1
C-1'	123.8	124.5	C-1'	120.9	121.1
C-2',6'	130.7	129.3	C-2',6'	130.9	131.1
C-3',5'	115.9	116.2	C-3',5'	115.2	115.3
C-4'	159.1	158.3	C-4'	160.1	160.1
7-OMe	56.2	56.0		56.0	56.1
3-O-glc			glc		
C-1''	100.8		C-1''	98.4	98.6
C-2''	74.2		C-2''	77.1 ^{a)}	77.4 ^{a)}
C-3''	76.4 ^{a)}		C-3''	76.4	76.4
C-4''	69.9		C-4''	70.3	70.3
C-5''	77.6		C-5''	77.7 ^{a)}	77.5 ^{a)}
C-6''	60.9		C-6''	60.8	60.8
4'-O-glc			api		
C-1'''	99.6	99.6	C-1'''	107.8	109.0
C-2'''	71.3	71.3	C-2'''	75.9	77.3 ^{a)}
C-3'''	76.9 ^{a)}	76.9 ^{a)}	C-3'''	77.6 ^{a)}	79.5
C-4'''	67.6	67.6	C-4'''	73.7	74.1
C-5'''	76.7 ^{a)}	76.8 ^{a)}	C-5'''	68.0	64.4
C-6'''	60.3	60.3			68.0
Terpene moiety			Aromatic moiety		
C-1	48.1	48.0	C-1	125.1	125.5
C-2	43.9	43.9	C-2	130.2	113.9
C-3	63.9	63.9	C-3	115.7	149.3
C-4	45.5	45.5	C-4	159.8	147.9
C-5	81.5	81.5	C-5	115.7	115.4
C-7	75.4	75.3	C-6	130.2	123.0
C-8	85.8	85.7	C-7	113.6	110.8
C-1'	135.7	135.6	C-8	144.4	144.6
C-2'	117.9	117.4	C-9	166.3	166.4
C-3'	150.4	150.4	3-OMe		55.6 ^{a)}
C-4'	129.6	129.6			
C-5'	164.6	164.6			
1-Me	16.1	16.1			
5-Me	19.6	19.5			
3'-Me	20.8	20.8			

Assignments with superscripts a) and b) in each vertical column may be interchanged.

pyranoside by the ^{13}C -NMR spectrum⁶⁾ (Table I). Furthermore, the location of the acyl group in **2** was determined at the C-5-OH of apiosyl residue in term of the acylation shifts [C-3, δ 77.6 (-1.9); C-5, δ 68.0 ($+3.6$)]. On the basis of these data, the full structure of **2** was elucidated as 3-O-[5'''-O-*p*-coumaroyl- β -D-apiofuranosyl-(1''' \rightarrow 2'')- β -D-glucopyranosyl] rhamnocitrin.

Compound **3**, a pale yellow powder, $[\alpha]_{\text{D}}^{25} -151.1^\circ$ (MeOH), $\lambda_{\text{max}}^{\text{MeOH}}$ (nm) 268 (log ϵ 4.46), 330 (log ϵ 4.52), showed peaks at m/z 793 due to $[\text{M} + \text{Na}]^+$ and at 301 due to $[\text{aglycone} + \text{H}]^+$ in the positive FAB-MS spectrum. The ^1H -NMR signals (in $\text{DMSO}-d_6$) due to the flavonol glycosidic residue at δ 12.53 (1H, s, 5-OH), 10.31 (1H, s, 4'-OH), 8.04 (2H, d, $J=8.8$ Hz, 2', 6'-H), 6.89 (2H, d, $J=8.8$ Hz, 3', 5'-H), 6.41 (1H, d, $J=2.2$ Hz, 8-H), 6.28 (1H, d, $J=2.2$ Hz, 6-H), 5.70 (1H, d, $J=7.3$ Hz, glc 1''-H), 5.40 (1H, s, api 1'''-H), 3.75 (3H, s, 7-OMe) indicated that the flavonol glycosidic moiety and location of acyl group

Chart 1. Structures of Compounds **1**, **2** and **3**

of **3** were the same as that of **2**, whose structure was also substantiated by the ^{13}C -NMR spectrum (Table I). Moreover, the signals due to the aromatic portion [δ 9.63 (1H, s, 4'-OH), 7.17 (1H, d, $J=15.4$ Hz, 7-H), 7.07 (1H, d, $J=1.5$ Hz, 2-H), 6.86 (1H, dd, $J=1.5, 8.1$ Hz, 6-H), 6.76 (1H, d, $J=8.1$ Hz, 5-H), 6.14 (1H, d, $J=16.1$ Hz, 8-H), 3.80 (3H, s, 3-OMe)] suggested the presence of a feruloyl group, which was also confirmed by observation of the FAB-MS peak due to $[\text{M} + \text{Na}]^+$ being 30 mass units higher than **2**. The NOE experiment and ^{13}C -NMR spectrum (Table I) also supported this proposed structure, so that the full structure of **3** was characterized as 3-O-[5'''-O-feruloyl- β -D-apiofuranosyl(1''' \rightarrow 2'')- β -D-glucopyranosyl] rhamnocitrin.

Experimental

Optical rotations were measured on a JASCO DIP-360 automatic digital polarimeter. The IR spectra were recorded with a Hitachi IR spectrometer, model 270-30. The ^1H - and ^{13}C -NMR spectra were measured with a JEOL JNM-GX 400 NMR spectrometer and chemical shifts are given on a δ (ppm) scale with tetramethylsilane (TMS) as an internal standard. The FAB- and EI-MS were recorded with a JEOL DX-303 HF spectrometer and taken in a glycerol matrix containing NaI. Thin layer chromatography (TLC) was performed on precoated Kieselgel 60 F₂₅₄ plate (0.2 mm Merck) and detection was achieved by spraying 10% H_2SO_4 followed by heating. Column chromatography was carried out with Sephadex LH-20 (Pharmacia Co., Ltd.), Bondapak C₁₈ (Waters Associates) and Kieselgel 60 (70–230 and 230–400 mesh, Merck).

Extraction and Separation Dried seeds (4.5 kg) of *Astragalus complanatus* collected in their natural habitat in China were extracted with MeOH and the extract (371 g) was partitioned between *n*-hexane and 80% MeOH. The 80% MeOH extract was further partitioned with 1-BuOH and water. The 1-BuOH soluble portion (90 g) was subjected to Sephadex LH-20 column chromatography with water and 10% MeOH \rightarrow MeOH to afford several fractions. The aromatic fraction (28 g) was chromatographed on MCI gel CHP 20P column with water and 10% MeOH \rightarrow MeOH and then fourteen fractions were obtained based upon TLC monitoring followed by column chromatography on Bondapak C₁₈ and silica gel to provide compounds **1** (31 mg), **2** (64 mg) and **3** (52 mg).

Compound **1**: A pale yellow powder, $[\alpha]_D^{25} -33.8^\circ$ (MeOH). IR ν_{\max}^{KBr} (cm^{-1}): 3432 (OH), 1660 (α,β -unsaturated carboxylic ester), 1602 (aromatic ring). UV $\lambda_{\max}^{\text{MeOH}}$ (nm): 267 (log ϵ , 4.59), 345 (log ϵ , 4.42). *Anal.* Calcd for $\text{C}_{43}\text{H}_{52}\text{O}_{20} \cdot 2\text{H}_2\text{O}$: C, 55.84; H, 6.10. Found: C, 55.84; H, 6.18. FAB-MS m/z : 889 $[\text{M}+\text{H}]^+$, 301 $[\text{aglycone}+\text{H}]^+$.

Enzymatic Hydrolysis of 1 A solution of **1** (25 mg) in acetate buffer (pH=4.2, 12 ml) was incubated with glycosidase at 37°C for 38 h and the hydrolysate was extracted with EtOAc. The organic layer was evaporated to dryness and the residue was chromatographed on silica gel $[\text{CHCl}_3\text{-MeOH-H}_2\text{O}$ (9:1:0.1 \rightarrow 8:2:0.2)] to provide **1a** (9 mg) as yellow amorphous powder, $[\alpha]_D^{25} +54.3^\circ$ (MeOH). FAB-MS m/z : 727 $[\text{M}+\text{H}]^+$, 301 $[\text{aglycone}+\text{H}]^+$. $^1\text{H-NMR}$ (in pyridine- d_5), flavonol moiety δ : 8.49 (2H, d, $J=8.8\text{ Hz}$, 2',6'-H), 7.50 (2H, d, $J=8.8\text{ Hz}$, 3',5'-H), 6.72 (1H, d, $J=2.2\text{ Hz}$, 8-H), 6.61 (1H, d, $J=2.2\text{ Hz}$, 6-H), 3.80 (3H, s, 7-OMe); glucosyl moiety δ : 6.07 (1H, dd, $J=9.2, 9.5\text{ Hz}$, 3-H), 5.83 (1H, d, $J=8.1\text{ Hz}$, 1-H), 4.55 (1H, dd, $J=9.2, 9.5\text{ Hz}$, 4-H), 4.50 (1H, d, $J=12.1\text{ Hz}$, 6-H_a), 4.46 (1H, dd, $J=8.1, 9.2\text{ Hz}$, 2-H), 4.44 (1H, d, $J=12.1\text{ Hz}$, 6-H_b), 4.24 (1H, overlapped, 5-H); terpene moiety δ : 8.86 (1H, d, $J=15.8\text{ Hz}$, 1'-H), 6.88 (1H, d, $J=16.1\text{ Hz}$, 2'-H), 5.84 (1H, s, 4'-H), 4.73 (1H, m, 3-H), 4.23 (1H, d, $J=7.3\text{ Hz}$, 7-H_a), 3.94 (1H, d, $J=7.3\text{ Hz}$, 7-H_b), 2.53 (1H, dd, $J=13.6, 7.0\text{ Hz}$, 2-H_a), 2.28 (1H, dd, $J=13.6, 10.3\text{ Hz}$, 4-H_a), 2.18 (2H, m, 2-H_b, 4-H_b), 1.81 (3H, s, 3'-Me), 1.53 (3H, s, 5-Me), 1.19 (3H, s, 1-Me) and $^1\text{H-}^1\text{H COSY}$. $^{13}\text{C-NMR}$ (DMSO- d_6) is shown in Table I.

Compound **2**: A pale yellow powder, $[\alpha]_D^{25} -148.4^\circ$ (MeOH). IR ν_{\max}^{KBr} (cm^{-1}): 3436 (OH), 1660 (conjugated carbonyl), 1602 (aromatic ring). UV $\lambda_{\max}^{\text{MeOH}}$ (nm): 267 (log ϵ , 4.58), 317 (log ϵ , 4.12). *Anal.* Calcd for $\text{C}_{36}\text{H}_{36}\text{O}_{17} \cdot 2\text{H}_2\text{O}$: C, 55.67; H, 5.19. Found: C, 55.55; H, 5.11. FAB-MS m/z : 763 $[\text{M}+\text{Na}]^+$, 301 $[\text{aglycone}+\text{H}]^+$. $^1\text{H-NMR}$ (DMSO- d_6) δ : 12.53 (1H, s), 10.56 (1H, s), 9.62 (1H, s), 8.04 (2H, d, $J=8.8\text{ Hz}$), 7.31 (2H, d, $J=8.8\text{ Hz}$), 7.19 (1H, d, $J=15.8\text{ Hz}$), 6.89 (2H, d, $J=8.8\text{ Hz}$), 6.76 (2H, d, $J=8.8\text{ Hz}$), 6.44 (1H, d, $J=2.2\text{ Hz}$), 6.28 (1H, d, $J=2.2\text{ Hz}$), 6.06 (1H, d, $J=16.1\text{ Hz}$), 5.69 (1H, d, $J=7.7\text{ Hz}$), 5.39 (1H, s), 3.76 (3H,

s) and $^1\text{H-}^1\text{H COSY}$. $^{13}\text{C-NMR}$ (DMSO- d_6) is shown in Table I.

Saponification of 2 Compound **2** (50 mg) was saponified with 3% KOH/MeOH in the usual manner and the product was chromatographed over silica gel $[\text{CHCl}_3\text{-MeOH-H}_2\text{O}$ (9:1:0.1 \rightarrow 8:2:0.2)] to provide **2a** (27 mg), a yellow amorphous powder, $[\alpha]_D^{25} -39.7^\circ$ (MeOH). FAB-MS m/z : 617 $[\text{M}+\text{Na}]^+$, 595 $[\text{M}+\text{H}]^+$, 301 $[\text{aglycone}+\text{H}]^+$. $^{13}\text{C-NMR}$ (DMSO- d_6) is shown in Table I.

Compound **3**: A pale yellow powder, $[\alpha]_D^{25} -151.1^\circ$ (MeOH). IR ν_{\max}^{KBr} (cm^{-1}): 3444 (OH), 1660 (conjugated carbonyl), 1606 (aromatic ring). UV $\lambda_{\max}^{\text{MeOH}}$ (nm): 268 (log ϵ , 4.46), 330 (log ϵ , 4.52). *Anal.* Calcd for $\text{C}_{37}\text{H}_{38}\text{O}_{18} \cdot 3/2\text{H}_2\text{O}$: C, 55.70; H, 5.18. Found: C, 55.49; H, 5.02. FAB-MS m/z : 793 $[\text{M}+\text{Na}]^+$, 301 $[\text{aglycone}+\text{H}]^+$. $^{13}\text{C-NMR}$ (DMSO- d_6) is shown in Table I.

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