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‴→2″)-β-D-glucopyranosyl] rhamnocitrin and 3-*O*-[5‴-*O*-feruloyl-β-D-apiofuranosyl(1‴→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 glycosides[3)] and a novel acylated flavonoid glycoside called complanatin[4)] 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° (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 λ_{max}^{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 (^{1}H-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 (^{13}C-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 ^{1}H-NMR signals were assignable to dihydrophaseic acid[5)] 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 $NaBH_4$-reductive product of phaseic acid. In addition, the ^{13}C-NMR spectrum (Table I) also suggested the occurrence of dihydrophaseic acid moiety. On the other hand, enzymatic hydrolysis of **1** afforded a deglucosyl product **1a** which showed a peak at *m/z* 727 due to $[M+H]^+$ in the positive FAB-MS spectrum. The ^{13}C-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 moietyl 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 ^{1}H-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 ^{1}H–^{1}H 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° (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 λ_{max}^{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 ^{1}H- and ^{13}C-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 ^{1}H-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 ^{13}C-NMR spectrum.[8)] Compound **2**, on saponification, provided a product **2a**, a pale yellow solid, $[\alpha]_D^{25}$ −39.7° (MeOH). The peak at *m/z* 595 resulting from $[M+H]^+$ in the positive FAB-MS spectrum and the ^{1}H-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→2)-β-D-gluco-

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

	1	**1a**		**2**	**2a**	**3**
Flavonol moiety			Flavonol moiety			
C-2	156.4	146.4	C-2	155.9	156.1	155.9
C-3	134.0	136.6	C-3	133.1	133.4	133.1
C-4	177.7	176.2	C-4	177.4	177.7	177.4
C-5	161.0	160.4	C-5	161.0	161.1	160.1
C-6	98.0	97.5	C-6	97.8	97.9	97.8
C-7	165.3	165.1	C-7	164.9	165.2	164.9
C-8	92.4	92.1	C-8	92.0	92.2	91.9
C-9	155.9	156.2	C-9	156.1	156.3	156.1
C-10	105.2	104.1	C-10	105.1	105.1	105.1
C-1′	123.8	124.5	C-1′	120.9	121.1	120.9
C-2′,6′	130.7	129.3	C-2′,6′	130.9	131.1	130.8
C-3′,5′	115.9	116.2	C-3′,5′	115.2	115.3	115.2
C-4′	159.1	158.3	C-4′	160.1	160.1	160.1
7-OMe	56.2	56.0		56.0	56.1	55.9[a]
3-*O*-glc			glc			
C-1″	100.8		C-1″	98.4	98.6	98.4
C-2″	74.2		C-2″	77.1[a]	77.4[a]	77.1
C-3″	76.4[a]		C-3″	76.4	76.4	76.4
C-4″	69.9		C-4″	70.3	70.3	70.3
C-5″	77.6		C-5″	77.7[a]	77.5[a]	77.7[b]
C-6″	60.9		C-6″	60.8	60.9	60.8
4′-*O*-glc			api			
C-1‴	99.6	99.6	C-1‴	107.8	109.0	107.7
C-2‴	71.3	71.3	C-2‴	75.9	77.3[a]	75.8
C-3‴	76.9[a]	76.9[a]	C-3‴	77.6[a]	79.5	77.6[b]
C-4‴	67.6	67.6	C-4‴	73.7	74.1	73.7
C-5‴	76.7[a]	76.8[a]	C-5‴	68.0	64.4	68.0
C-6‴	60.3	60.3				
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.

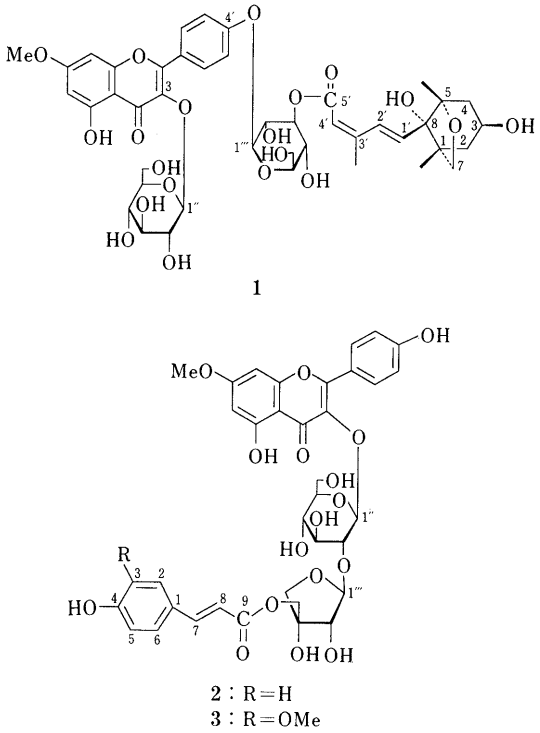

Chart 1. Structures of Compounds **1**, **2** and **3**

pyranoside by the ^{13}C-NMR spectrum[6] (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‴→2″)-β-D-glucopyranosyl] rhamnocitrin.

Compound **3**, a pale yellow powder, $[\alpha]_D^{25}$ −151.1° (MeOH), λ_{max}^{MeOH} (nm) 268 (log ε 4.46), 330 (log ε 4.52), showed peaks at *m/z* 793 due to $[M+Na]^+$ and at 301 due to $[aglycone+H]^+$ in the positive FAB-MS spectrum. The ^{1}H-NMR signals (in 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 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 $[M+Na]^+$ 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‴→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 ^{1}H- 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 F_{254} 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 C_{18} (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 furthr 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→MeOH to afford several fractions. The aromatic fraction (28 g) was chromatographed on MCI gel CHP 20P column with water and 10% MeOH→MeOH and then fourteen fractions were obtained based upon TLC monitoring followed by column chromatography on Bondapak C_{18} 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° (MeOH). IR ν_{max}^{KBr} (cm^{-1}): 3432 (OH), 1660 (α,β-unsaturated carboxylic ester), 1602 (aromatic ring). UV λ_{max}^{MeOH} (nm): 267 (log ε, 4.59), 345 (log ε, 4.42). *Anal.* Calcd for $C_{43}H_{52}O_{20}\cdot 2H_2O$: C, 55.84; H, 6.10. Found: C, 55.84; H, 6.18. FAB-MS *m/z*: 889 $[M+H]^+$, 301 $[aglycone+H]^+$.

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 [$CHCl_3$–MeOH–H_2O (9 : 1 : 0.1→8 : 2 : 0.2)] to provide **1a** (9 mg) as yellow amorphous powder, $[\alpha]_D^{25}$ +54.3° (MeOH). FAB-MS *m/z*: 727 $[M+H]^+$, 301 $[aglycone+H]^+$. ^{1}H-NMR (in pyridine-d_5), flavonol moiety δ: 8.49 (2H, d, *J* = 8.8 Hz, 2′,6′-H), 7.50 (2H, d, *J* = 8.8 Hz, 3′,5′-H), 6.72 (1H, d, *J* = 2.2 Hz, 8-H), 6.61 (1H, d, *J* = 2.2 Hz, 6-H), 3.80 (3H, s, 7-OMe); glucosyl moiety δ: 6.07 (1H, dd, *J* = 9.2, 9.5 Hz, 3-H), 5.83 (1H, d, *J* = 8.1 Hz, 1-H), 4.55 (1H, dd, *J* = 9.2, 9.5 Hz, 4-H), 4.50 (1H, d, *J* = 12.1 Hz, 6-H_a), 4.46 (1H, dd, *J* = 8.1, 9.2 Hz, 2-H), 4.44 (1H, d, *J* = 12.1 Hz, 6-H_b), 4.24 (1H, overlapped, 5-H); terpene moiety δ: 8.86 (1H, d, *J* = 15.8 Hz, 1′-H), 6.88 (1H, d, *J* = 16.1 Hz, 2′-H), 5.84 (1H, s, 4′-H), 4.73 (1H, m, 3-H), 4.23 (1H, d, *J* = 7.3 Hz, 7-H_a), 3.94 (1H, d, *J* = 7.3 Hz, 7-H_b), 2.53 (1H, dd, *J* = 13.6, 7.0 Hz, 2-H_a), 2.28 (1H, dd, *J* = 13.6, 10.3 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}H–^{1}H COSY. ^{13}C-NMR (DMSO-d_6) is shown in Table I.

Compound **2**: A pale yellow powder, $[\alpha]_D^{25}$ −148.4° (MeOH). IR ν_{max}^{KBr} (cm^{-1}): 3436 (OH), 1660 (conjugated carbonyl), 1602 (aromatic ring). UV λ_{max}^{MeOH} (nm): 267 (log ε, 4.58), 317 (log ε, 4.12). *Anal.* Calcd for $C_{36}H_{36}O_{17}\cdot 2H_2O$: C, 55.67; H, 5.19. Found: C, 55.55; H, 5.11. FAB-MS *m/z*: 763 $[M+Na]^+$, 301 $[aglycone+H]^+$. ^{1}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 Hz), 7.31 (2H, d, *J* = 8.8 Hz), 7.19 (1H, d, *J* = 15.8 Hz), 6.89 (2H, d, *J* = 8.8 Hz), 6.76 (2H, d, *J* = 8.8 Hz), 6.44 (1H, d, *J* = 2.2 Hz), 6.28 (1H, d, *J* = 2.2 Hz), 6.06 (1H, d, *J* = 16.1 Hz), 5.69 (1H, d, *J* = 7.7 Hz), 5.39 (1H, s), 3.76 (3H, s) and ^{1}H–^{1}H COSY. ^{13}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 [$CHCl_3$–MeOH–H_2O (9 : 1 : 0.1→8 : 2 : 0.2)] to provide **2a** (27 mg), a yellow amophous powder, $[\alpha]_D^{25}$ −39.7° (MeOH). FAB-MS *m/z*: 617 $[M+Na]^+$, 595 $[M+H]^+$, 301 $[aglycone+H]^+$. ^{13}C-NMR (DMSO-d_6) is shown in Table I.

Compound **3**: A pale yellow powder, $[\alpha]_D^{25}$ −151.1° (MeOH). IR ν_{max}^{KBr} (cm^{-1}): 3444 (OH), 1660 (conjugated carbonyl), 1606 (aromatic ring). UV λ_{max}^{MeOH} (nm): 268 (log ε, 4.46), 330 (log ε, 4.52). *Anal.* Calcd for $C_{37}H_{38}O_{18}\cdot 3/2H_2O$: C, 55.70; H, 5.18. Found: C, 55.49; H, 5.02. FAB-MS *m/z*: 793 $[M+Na]^+$, 301 $[aglycone+H]^+$. ^{13}C-NMR (DMSO-d_6) is shown in Table I.

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References and Notes

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