



Flavanone glycosides from *Alhagi pseudalhagi*

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Abstract

Two new flavanone glycosides, alhagitin and alhagidin, have been isolated from the whole plant of *Alhagi pseudalhagi* and their structures established respectively as naringenin 5-methyl ether 4'-glucoside and hesperitin 7-galactosyl(1 → 2)[rhamnosyl(1 → 6)]glucoside by chemical and spectroscopic methods. © 1999 Published by Elsevier Science Ltd. All rights reserved.

Keywords: *Alhagi pseudalhagi*; Leguminosae; Whole plant; Flavanone glycosides; Alhagitin; Alhagidin

1. Introduction

Alhagi pseudalhagi (Bieb.) Desv. (Leguminosae) is distributed throughout India, where it is used as traditional herbal medicine (Kirtikar, & Basu, 1984; Khushbaktova et al., 1992; Viramani et al., 1992). Phenolic constituents previously reported from this plant include: (+)-catechin, (–)-epigallocatechin, (±)-gallocatechin and leucodelphinidin (Islambekov, Mirzakhidov, Karimolzhano, & Ishbaev, 1982), quercetin (Khaitbaev, Sultan, Ganiev, & Aslanov, 1993) and rutin (Svistunova, Khalmatov, & Khazanovich, 1972). We report here the isolation of two new flavanone glycosides, alhagitin (**1**) and alhagidin (**2**), from the whole plant of *A. pseudalhagi*.

2. Results and discussion

Chromatographic resolution of the methanolic extract of the whole plant of *A. pseudalhagi* yielded the glycosides, alhagitin (**1**), C₂₂H₂₄O₁₀ and alhagidin (**2**), C₃₄H₄₄O₂₀. Both compounds developed a magenta color with Mg/HCl and exhibited UV absorption bands typical of flavanones (Imperato, 1978).

Alhagitin (**1**) showed peaks in its IR spectrum at

3000–3400 cm^{−1} (br) for a polyhydroxy system and at 1640 cm^{−1} for a conjugated carbonyl group. On acid hydrolysis, it gave glucose and aglycone (**3**) which furnished a diacetate (**4**). The ¹H NMR spectrum of **3** showed a methoxyl group signal (δ 3.78), a typical four peak pattern doublet for a 4'-oxygenated B-ring (δ 6.80, 7.30, *J* = 8.5 Hz, each), two meta coupled protons merged as a broad singlet (δ 5.91) and the same ABX pattern of protons as for naringenin 5-methyl ether (Maruyama, Hayasaka, & Sasaki, 1974). The mass spectrum showed a molecular ion peak at *m/z* 286.0846 and had characteristic ion peaks (*m/z* 166 and *m/z* 120) due to retro-Diel's-Alder type fragmentation indicating the presence of a methoxyl group in ring A. These data suggest that **3** is naringenin 5-methyl ether and was further corroborated by the ¹³C NMR data (Harborne, & Mabry, 1982) (Table 1), which also indicated the presence of only one sugar from the single anomeric carbon signal at δ 101.4. The attachment of a glucose unit at C-4' was apparent from the UV spectrum of **1** which showed a bathochromic shift of 10 nm in the presence of sodium acetate and no bathochromic shift with alkali. The structure of **1** is thus determined as naringenin 5-methyl ether 4'-glucoside which is a new flavanone glycoside.

Alhagidin (**2**) showed peaks in its IR spectrum at 3200–3500 cm^{−1} (br) for a polyhydroxy system, at 2910 cm^{−1} for a methoxyl group and at 1635 cm^{−1} for a conjugated carbonyl group. On acid hydrolysis, **2**

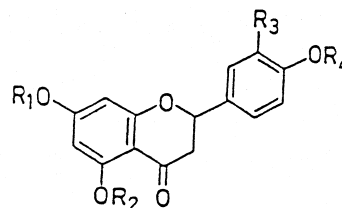
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Table 1
 ^{13}C NMR spectral data in δ value of compounds 1–3

Carbon number	1	2	3
C-2	78.2	78.49	78.2
C-3	42.2	42.13	42.0
C-4	196.2	197.17	196.0
C-5	163.1	163.10	163.0
C-6	96.0	96.48	95.8
C-7	166.1	165.17	166.0
C-8	95.0	95.65	94.8
C-9	163.0	163.15	162.8
C-10	102.0	103.42	101.8
C-1'	128.33	130.96	128.3
C-2'	115.1	114.24	115.0
C-3'	128.0	148.61	128.0
C-4'	157.1	146.52	157.0
C-5'	128.0	112.06	128.0
C-6'	115.1	118.11	115.0
C-1''	101.4	99.51	
C-2''	73.8	73.19	
C-3''	77.6	76.35	
C-4''	70.8	70.66	
C-5''	77.0	75.60	
C-6''	62.0	66.18	
C-1'''		100.70	
C-2'''		70.37	
C-3'''		69.55	
C-4'''		72.17	
C-5'''		68.43	
C-6'''		17.95	
C-1''''		92.69	
C-2''''		69.04	
C-3''''		68.88	
C-4''''		70.47	
C-5''''		69.68	
C-6''''		60.72	
-OCH ₃		55.76	

gave galactose, glucose and rhamnose (co-PC) and an aglycone (**5**). The aglycone (**5**) $\text{C}_{16}\text{H}_{14}\text{O}_6$, showed a methoxyl group signal (δ 3.70), four meta coupled protons, one ortho coupled proton and an ABX pattern of protons like that of 5,7,3'-trihydroxy-4'-methoxyflavanone (hesperitin) (Harborne, & Mabry, 1982). The mass spectrum showed a molecular ion peak at m/z 302 and had characteristic ion peaks (m/z 152 and 150) due to retro-Diel's-Alder type fragmentation indicating the presence of a methoxyl group in ring B. These data suggest that **5** is hesperitin. UV spectra of **2** showed a bathochromic shift of 22 nm with aluminum chloride but the absence of a shift with sodium acetate indicated the attachment of sugar residues at the C-7 position. ^1H and ^{13}C NMR of **2** showed respectively three anomeric protons (δ 5.20, 2H, br s and 5.45, 1H, m) and three anomeric carbons (δ 99.5, 100.6 and 92.6). FAB-MS exhibited a molecular ion peak at m/z 811 $[\text{M}^+ + \text{K}]^+$ which favoured the presence of one mole each of galactose, glucose and rhamnose. Permethylolation of **2** and hydrolysis of the

permethylate furnished 3,4-di-methylglucose, 2,3,4-trimethylrhamnose and 2,3,4,6-tetramethyl galactose. The structure of **2** is thus established as hesperitin 7-galactosyl(1 \rightarrow 2)[rhamnosyl(1 \rightarrow 6)]glucoside, which is a new flavanone glycoside, designated alhagidin.

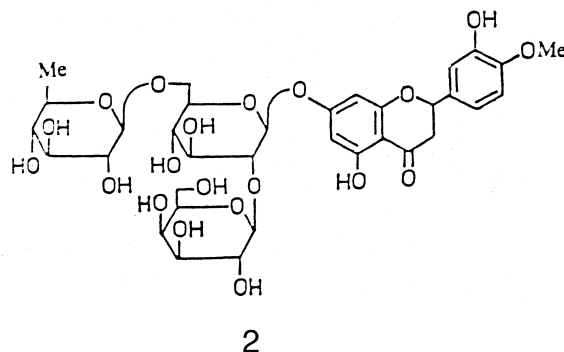


1 : $\text{R}_1 = \text{R}_3 = \text{H}$, $\text{R}_2 = \text{Me}$, $\text{R}_4 = \text{Glc}$.

3 : $\text{R}_1 = \text{R}_3 = \text{R}_4 = \text{H}$, $\text{R}_2 = \text{Me}$

4 : $\text{R}_1 = \text{R}_4 = \text{Ac}$, $\text{R}_2 = \text{Me}$, $\text{R}_3 = \text{H}$

5 : $\text{R}_1 = \text{R}_2 = \text{H}$, $\text{R}_3 = \text{OH}$, $\text{R}_4 = \text{Me}$



3. Experimental

Mps were uncorr. CC was carried out on silica gel (BDH, 60–120 mesh), TLC on silica gel and PC on Whatman No 1 paper. Solvents used for TLC were C_6H_6 – CHCl_3 (1:4, solvent A), CHCl_3 – MeOH (3:1, solvent B) and MeOH – H_2O (10:1, solvent C) and for PC: n - BuOH – HOAc – H_2O (4:1:5, solvent D). Liebermann–Burchard reagent was used for developing TLC plates. PCs were developed with acetic acid AgNO_3 – NaOH and washed with $\text{Na}_2\text{S}_2\text{O}_3$ solution. ^1H NMR and ^{13}C NMR spectra were recorded on 300 and 100 MHz Varian spectrometers, respectively. TMS was used as int. standard and chemical shift values were recorded in δ ppm. EIMS and FAB-MS were performed on a Kratos MS-50 instrument at 70 eV with evaporation of sample in the ion source. Whole plants of *A. pseudalhagi* were collected from the Varanasi District, U.P., India and the identification verified by the Department of Botany, Banaras Hindu University, Varanasi. A herbarium specimen is kept in the Department of Medicinal Chemistry, IMS, BHU.

The whole plant (3 kg) was dried, powdered and

repeatedly extracted with MeOH by cold percolation at 25°. The MeOH extract afforded a green semi-solid (60 g) which was chromatographed over a silica gel column eluting with solvents of increasing polarity. The eluants from C₆H₆–CHCl₃ (1:1), (1:2), CHCl₃, CHCl₃–MeOH (1:1) and (1:2) furnished, respectively, apigenin (30 mg), naringenin (35 mg), hesperidin (28 mg), alhagitin (**1**) (25 mg) and alhagidin (**2**) (36 mg).

3.1. Alhagitin (**1**)

Compound **1** crystallized from MeOH as light yellow granules, *R*_f 0.19 (solvent A), 0.45 (solvent B); molecular ion peak at *m/z* 449 as a cationized cluster ion [C₂₂H₂₄O₁₀ + H]⁺ (FAB–MS); IR ν_{\max} (KBr, cm^{−1}) 3000–3400, 1640, 1460, 1310, 1250, 1175, 1160, 1060; UV $\lambda_{\max}^{\text{MeOH}}$: 287 (log ϵ 4.26), 325 sh (log ϵ 3.16) nm; ¹³C NMR (DMSO-d₆): see Table 1.

3.2. Hydrolysis of alhagitin (**1**)

Compound **1** (28 mg) was dissolved in MeOH (10 ml) and H₂O (1 ml) and refluxed with H₂SO₄ (1 ml) for 5 h. The reaction mixture was poured into H₂O, the MeOH removed and the mixture extracted with CHCl₃. The CHCl₃ extract yielded naringenin 5-methyl ether (**3**) (17 mg) as colorless needles, mp 257–59°, C₁₆H₁₄O₅ (M⁺ 286.0846, HRMS); UV $\lambda_{\max}^{\text{MeOH}}$ 285, 322 sh nm; ¹H NMR (CCl₄, δ): 2.70 (2H, m, H-3), 3.78 (3H, s, -OCH₃), 5.40 (1H, m, H-2), 6.12 (1H, d, *J* = 2 Hz, H-6), 6.32 (1H, d, *J* = 2 Hz, H-8), 6.80 (2H, d, *J* = 8 Hz, H-2' and H-3'), 7.30 (2H, d, *J* = 8 Hz, H-5' and H-6'), 9.10 (1H, br s, 4'-OH, exchangeable with D₂O), 10.00 (1H, br, s, 7-OH, exchangeable with D₂O). ¹³C NMR (DMSO-d₆, δ): see Table 1. HRMS: *m/z* 286.0846 (M⁺, 100%), 286(30), 258(58), 179(10), 166(75), 138(65), 135(14), 134(16), 120(22), 119(26), 108(62), 98(24), 73(22), 57(22). On acetylation with Ac₂O/triethylamine, **3** furnished naringenin 5-methyl ether 7, 4'-diacetate (**4**) as colorless needles, mp 171–73°; IR ν_{\max} (KBr cm^{−1}): 1743, 1682; ¹H NMR (CDCl₃, δ): 2.25 (6H, s, 2 × OAc), 2.72 (2H, m, H-3), 3.70 (3H, s, -OCH₃), 5.42 (1H, m, H-2), 6.31 (1H, d, *J* = 2 Hz, H-6), 6.44 (1H, d, *J* = 2 Hz, H-8), 7.10 (2H, d, *J* = 8 Hz, H-2' and H-3'), 7.44 (2H, d, *J* = 8 Hz, H-5' and H-6'). Found: C, 64.66, H, 5.28% calcd. for C₂₀H₁₈O₇: C, 64.86, H, 4.90%. The hydrolysate showed a single spot on PC (solvent D) which corresponded to glucose (co-PC with authentic sample).

3.3. Alhagidin (**2**)

Compound **2** crystallized from MeOH as buff colored granules, *R*_f 0.34 (solvent C); molecular ion peak at *m/z* 811 as a cationized cluster ion

[C₃₄H₄₄O₂₀ + K]⁺ (FAB–MS); UV $\lambda_{\max}^{\text{MeOH}}$ 270 (log ϵ 4.19), 3.12 (log ϵ 3.78); + AlCl₃ 292 (log ϵ 4.15), 352 (log ϵ 3.00) nm; IR ν_{\max} (KBr, cm^{−1}): 3200–3540 (-OH), 2910 (-OCH₃), 1635, 1605 (>C=O), 1515, 1355, 1275, 1200, 1130, 1090; ¹H NMR (DMSO-d₆, δ): 1.10 (3H, d, *J* = 5 Hz, rhamnosyl CH₃), 2.78 (1H, dd, *J* = 2 Hz and 10.5 Hz, H-3), 3.15 (1H, m, H-3), 3.75 (3H, s, -OCH₃), 3.20–4.50 (complex, glucosyl, rhamnosyl and galactosyl protons), 5.20 (2H, br, s, one glucosyl and one rhamnosyl anomeric protons), 5.45 (2H, m, H-2 and one galactosyl anomeric proton), 6.15 (1H, d, *J* = 2.0 Hz, H-6), 6.17 (1H, d, *J* = 2 Hz, H-8), 6.80 (3H, m, H-2', H-5' and H-6'), 9.15 (1H, br, s, 3'-OH), 12.0 (1H, br, s, 5-OH). ¹³C NMR (DMSO-d₆, δ): see Table 1.

3.4. Hydrolysis of alhagidin (**2**)

Compound **2** (30 mg) was dissolved in MeOH (12 ml) and H₂O (2 ml) and refluxed with H₂SO₄ (1 ml) for 6 h. The reaction mixture was worked up in the usual manner and extracted with CHCl₃. The CHCl₃ extract yielded hesperitin (**5**) as cream colored granules, mp 132–36°; C₁₆H₁₄O₆ (M⁺, 302); UV $\lambda_{\max}^{\text{MeOH}}$ 275 (log ϵ 4.00), 303 (log ϵ 3.12) nm; ¹H NMR (DMSO-d₆, δ): 2.77 (2H, m, H-3), 3.70 (3H, s, -OCH₃), 5.50 (1H, m, H-2), 6.19 (1H, d, *J* = 2 Hz, H-6), 6.24 (1H, d, *J* = 2 Hz, H-8), 6.89 (3H, m, H-2', H-5' and H-6'), 9.00 (1H, br, s, 7-OH, exchangeable with D₂O), 11.90 (1H, br, s, 5-OH, exchangeable with D₂O). MS: *m/z* 302 (M⁺), 274, 273, 179, 152, 150, 124, 120, 98, 97. The aqueous hydrolysate showed three spots on PC (solvent D) which corresponded to galactose, glucose and rhamnose (co-PC with authentic samples).

3.5. Methylation of alhagidin (**2**)

Compound **2** was methylated using DMSO according to the Hakomori method (Hakomori, 1964). Alhagidin (**2**) (25 mg) was treated with NaH (70 mg) and MeI (1.5 ml) in DMSO (8 ml) in N₂. The reaction mixture was diluted with H₂O and extracted with CHCl₃ in the usual way. The methylated product on purification by prep. TLC gave a semi solid mass, *R*_f 0.20 (solvent B), which showed no hydroxyl absorption in its IR. The permethylated product was refluxed with 6% HCl in MeOH for 1 h. The MeOH was removed from the reaction mixture which was then extracted with CHCl₃. The CHCl₃ extract could not be purified because of excess amount of coloring matter and the small amount of aglycone. The aqueous hydrolysate was concentrated and co-PC with authentic methylated sugars indicated the presence of 3,4-di-methylglucose: 2,3,4-tri-methylrhamnose and 2,3,4,6-tetra-methyl galactose (solvent D).

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