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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.

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, Karimolzhanov, & Ishbaev, 1982), quercetin (Khaiitbaev, 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**), $C_{22}H_{24}O_{10}$ and alhagidin (**2**), $C_{34}H_{44}O_{20}$. 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 ^{13}C 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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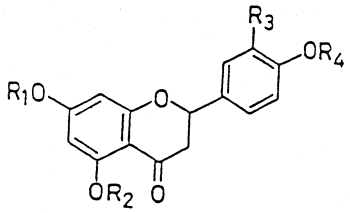
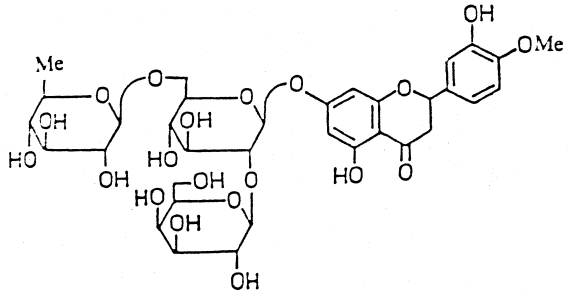
Table 1
^{13}C NMR spectral data in 'δ' value of compounds **1**–**3**

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	
-O*C*H$_3$		55.76	

gave galactose, glucose and rhamnose (co-PC) and an aglycone (**5**). The aglycone (**5**) $C_{16}H_{14}O_6$, 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 $[M^+ + K]^+$ which favoured the presence of one mole each of galactose, glucose and rhamnose. Permethylation 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 → 2)[rhamnosyl(1 → 6)glucoside, which is a new flavanone glycoside, designated alhagidin.



1 : $R_1 = R_3 = H$, $R_2 = Me$, $R_4 = Glc.$
3 : $R_1 = R_3 = R_4 = H$, $R_2 = Me$
4 : $R_1 = R_4 = Ac$, $R_2 = Me$, $R_3 = H$
5 : $R_1 = R_2 = H$, $R_3 = OH$, $R_4 = Me$



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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 acetonic $AgNO_3$–NaOH and washed with $Na_2S_2O_3$ 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 C_6H_6–$CHCl_3$ (1:1), (1:2), $CHCl_3$, $CHCl_3$–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 $[C_{22}H_{24}O_{10}+H]^+$ (FAB–MS); IR ν_{max} (KBr, cm^{-1}) 3000–3400, 1640, 1460, 1310, 1250, 1175, 1160, 1060; UV_{max}^{MeOH}: 287 (log ε 4.26), 325 sh (log ε 3.16) nm; ^{13}C NMR (DMSO-d6): see Table 1.

3.2. Hydrolysis of alhagitin (*1*)

Compound **1** (28 mg) was dissolved in MeOH (10 ml) and H_2O (1 ml) and refluxed with H_2SO_4 (1 ml) for 5 h. The reaction mixture was poured into H_2O, the MeOH removed and the mixture extracted with $CHCl_3$. The $CHCl_3$ extract yielded naringenin 5-methyl ether (**3**) (17 mg) as colorless needles, mp 257–59°, $C_{16}H_{14}O_5$ (M^+ 286.0846, HRMS); UV λ_{max}^{MeOH} 285, 322 sh nm: ^1H NMR (CCl_4, δ): 2.70 (2H, m, H-3), 3.78 (3H, s, -OCH_3), 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 D_2O), 10.00 1H, br, s, 7-OH, exchangeable with D_2O). ^{13}C NMR (DMSO-d_6, 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 Ac_2O/triethylamine, **3** furnished naringenin 5-methyl ether 7, 4′-diacetate (**4**) as colorless needless, mp 171–73°; IR ν_{max} (KBr cm^{-1}): 1743, 1682; ^1H NMR ($CDCl_3$, δ): 2.25 (6H, s, 2 × OAc), 2.72 (2H, m, H-3), 3.70 (3H, s, -OCH_3), 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 $C_{20}H_{18}O_7$: 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 $[C_{34}H_{44}O_{20}+K]^+$ (FAB–MS); UV λ_{max}^{MeOH} 270 (log ε 4.19), 3.12 (log ε 3.78); $+AlCl_3$ 292 (log ε 4.15), 352 (log ε 3.00) nm; IR ν_{max} (KBr, cm^{-1}): 3200–3540 (-OH), 2910 (-OCH_3), 1635, 1605 (>C=0), 1515, 1355, 1275, 1200, 1130, 1090; ^1H NMR (DMSO-d_6, δ): 1.10 (3H, d, $J=5$ Hz, rhamnosyl CH_3), 2.78 (1H, dd, $J=2$ Hz and 10.5 Hz, H-3), 3.15 (1H, m, H-3), 3.75 (3H, s, -OCH_3), 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). ^{13}C NMR (DMSO-d_6, δ): see Table 1.

3.4. Hydrolysis of alhagidin (*2*)

Compound **2** (30 mg) was dissolved in MeOH (12 ml) and H_2O (2 ml) and refluxed with H_2SO_4 (1 ml) for 6 h. The reaction mixture was worked up in the usual manner and extracted with $CHCl_3$. The $CHCl_3$ extract yielded hesperitin (**5**) as cream colored granules, mp 132–36°; $C_{16}H_{14}O_6$ (M^+, 302); UV λ_{max}^{MeOH} 275 (log ε 4.00), 303 (log ε 3.12) nm; ^1H NMR (DMSO-d_6, δ): 2.77 (2H, m, H-3), 3.70 (3H, s, -OCH_3), 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 D_2O), 11.90 (1H, br, s, 5-OH, exchangeable with D_2O. 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 N_2. The reaction mixture was diluted with H_2O and extracted with $CHCl_3$ 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 $CHCl_3$. The $CHCl_3$ 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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