NEW OLIGOMERIC PROANTHOCYANIDINS FROM *Alhagi pseudalhagi*

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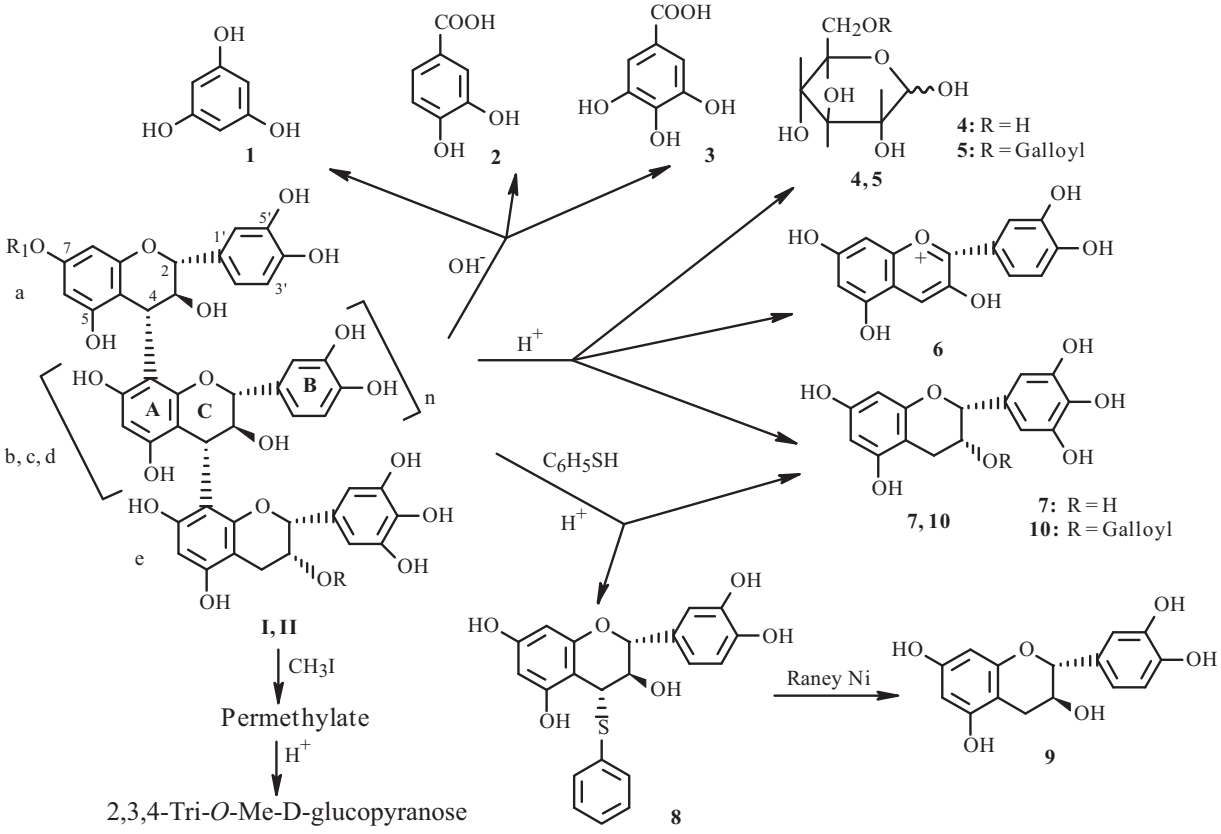
Two new oligomeric proanthocyanidin glucosides were isolated from the aerial part and roots of Alhagi pseudalhagi. *Their structures and relative configurations were elucidated as 7-*O-β-*D-Glc*p-(6→)-*galloyl-(+)-catechin-(4α-8)-(+)-catechin-(4α-8)-(–)-epigallocatechin and 7-*O-β-*D-Glc*p-(6→)-*galloyl-(+)-catechin-[(4α-8)-(+)-catechin]3-(4α-8)-(–)-epigallocatechin-3-*O-*gallate.*

Keywords: *Alhagi pseudalhagi*, Fabaceae, oligomeric proanthocyanidins, isolation, structure, NMR spectroscopy.

We isolated 12 pure compounds from the total extractable compounds in the aqueous alcohol extract of *Alhagi pseudalhagi* (Bieb.) Fisch by using column chromatography over silica gel and microcrystalline cellulose in addition to gel chromatography over Sephadex LH-20 [1]. Of these, six compounds were identified as monomeric catechins; four, known dimeric proanthocyanidins [2]. Two compounds were believed to be oligomeric acylglycosylated proanthocyanidins that we called alhacin (**I**) and alhacidin (**II**) on the basis of UV and IR spectra.



I: R = H, R_1 = Glc*p*-(6→)Galloyl; n = 1; **II:** R = Galloyl, R_1 = Glc*p*-(6→)Galloyl; n = 3

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TABLE 1. Chemical Shifts of Resonances in the ^{13}C NMR Spectrum of **I** (ppm)

C atom	δ_C of fragments				
	a	b	e	Glucose	Galloyl
C-2	83.41	81.84	79.31		
C-3	73.54[a]	73.81[a]	65.41		
C-4	38.81	38.81	–		
C-6	96.03	96.03	96.03		
C-8	96.03	107.44	107.44		
C-10	102.74	102.74	102.74		
C-5, 7, 9	153.84–155.50	153.84–155.50	153.84–155.50		
C-1′	130.64	130.64	130.64	102.74	119.91
C-2′	114.94	114.94	109.61[b]	72.81[a]	109.61[b]
C-3′	143.83	143.83	145.02	77.34	145.02
C-4′	143.83	143.83	133.80	70.52	138.84
C-5′	115.21	115.21	145.02	75.53	145.02
C-6′	118.40	118.40	109.61[b]	63.10	109.01[b]
-COO-					168.38

Resonances denoted by the same superscripts may be reversed.

The proanthocyanidin alhacin was optically active, $[\alpha]_D^{22}$ +13.2° (*c* 0.37, EtOH), and decomposed at 290–300°C without melting. Its molecular weight (MW ~ 1210–1220) and composition ($C_{58}H_{52}O_{29}$) were determined by sedimentation equilibrium in an ultracentrifuge. Gel filtration over a calibrated column of Sephadex LH-20 confirmed these results.

Several chemical transformations were carried out in order to determine the monomer composition and elucidate the structure. Alkaline cleavage of alhacin under a N_2 atmosphere formed three compounds that were identified using physicochemical properties as phloroglucinol (**1**) and protocatechoic (**2**) and gallic (**3**) acids [3–5].

Acid hydrolysis of alhacin formed (-)-epigallocatechin (**7**), cyanidin (**6**), *β*-glucose (**4**), and galloylglucose (**5**) [6, 7].

Mild thiolytic cleavage in the presence of thiophenol and acetic acid produced from the "lower" block of alhacin (–)-epigallocatechin (**7**); from the "upper" block, thioether **8**, which was destroyed catalytically in the presence of Raney nickel [8]. The resulting compound was identified as (+)-catechin (**9**).

Thus, the chemical analysis showed that proanthocyanidin alhacin was a glycosylated oligomeric proanthocyanidin consisting of (+)-catechin and (–)-epigallocatechin.

In fact, the ^{13}C NMR spectrum (Table 1) of alhacin that was taken with full proton decoupling exhibited resonances characteristic of epigallocatechin, catechin, *β*-glucose, and gallic acid. A broad resonance at 153.84–155.50 ppm was assigned to C-5, C-7, and C-9. A resonance at 96.03 ppm belonged to unsubstituted C-6 and C-8 of phloroglucinol. Resonances at 109.01 and 109.61 ppm (C-2′ and C-6′), 145.03 (C-3′ and C-5′), and 133.80 (C-4′) were indicative of a gallocatechin block whereas resonances at 114.94, 115.21, 118.40, and 143.84 were assigned to C-2′, C-5′, C-6′ and C-3′, and C-4′, respectively, and showed that alhacin had a catechin block. Resonances at about 81-83 (C-2) showed that the upper blocks of the proanthocyanidin had the 2,3-*trans* orientation [6-13].

It is known that the resonance for C-10 appears at greater than 100.0 ppm if C-4–C-8 interflavane bonds are present. For C-4–C-6 interflavane bonds, this resonance shifts to even stronger field [14].

Based on our results (chemical shift of C-10 of 102.74 ppm), it was found that alhacin had C-4–C-8 interflavane bonds.

Resonances for glucose C-1, C-3, and C-5 at 102.74, 77.34, and 75.53 ppm, respectively, showed that the anomeric center had the *β*-configuration. A resonance at 63.10 for a substituted glucose C-6 proved that the carbohydrate was acylated in the 6-position [15, 16].

A D-glucose acylated by gallic acid in the 6-position was bonded to the aglycon by a *β*-glycoside bond. Mild thiolytic cleavage of alhacin was consistent with the lack of a sugar in the lower block of the molecule. Considering this and the steric hindrance in the "middle" block of the proanthocyanidin, we hypothesized that the most likely site of addition of the acylated glucose was C-7 of the upper block.

TABLE 2. Chemical Shifts of Resonances in the ^{13}C NMR Spectrum of **II** (ppm)

C atom	δ_C of fragments				
	a	b, c, d	e	Glucose	Galloyl
C-2	83.11	81.63–82.34	77.01		
C-3	73.21*	72.80*	69.64		
C-4	38.44	38.44	26.70		
C-6	96.70	96.70	96.70		
C-8	96.70	107.63	107.63		
C-10	102.30	102.30	102.30		
C-5, 7, 9	154.20	154.20	154.20		
C-1′	131.01	130.01	131.01	102.30	121.73
C-2′	114.63[a]	115.14[a]	108.94	73.21	109.83
C-3′	144.33	144.33	144.33	77.01	144.33
C-4′	144.33	144.33	133.60	71.72	139.10
C-5′	115.90	115.90	144.33	75.82	144.33
C-6′	119.10	119.10	108.94	63.20	109.83
-COO-					167.21

[a]Resonances denoted by the same superscripts may be reversed.
*Resonance not observed because of overlap with the solvent resonance.

Thus, the chemical transformations and the physicochemical and spectral data reported above suggested for alhacin the structure and relative configuration 7-*O*-β-D-Glc*p*$\xrightarrow{6}$galloyl-(+)-catechin-(4α-8)-(+)-catechin-(4α-8)-(–)-epigallocatechin.

Proanthocyanidin alhacidin (**II**) was optically active, $[\alpha]_D^{22}$ +21.9° (*c* 0.29, EtOH); mp 290–300°C (dec.), MW = 1935–1945, $C_{95}H_{80}O_{45}$. The physicochemical properties and spectral parameters (UV, IR, ^{13}C NMR) of **II** (Table 2) were very similar to those of **I**. The difference was that **II** had a higher molecular weight. Based on this, proanthocyanidin **II** also consisted of (+)-catechin and (–)-epigallocatechin blocks and had an acylated glucose.

The ^{13}C NMR of **II** had resonances at 77.01 and 26.70 ppm for C-2 and C-4 of the heterocycle. This showed that the lower block had a galloyl substituent in the C-3 position. There was also a C-4–C-8 interflavane bond (CS of C-10, 102.30 ppm). Chemical shifts of C-2 and C-4 of the upper and middle blocks indicated that **II** had the 2,3-*trans* configuration and lacked an ester bond in the C-3 position [17].

The most probable location of the acylated glucose was C-7 of the upper block of **II** according to the same data as for **I**.

Both proanthocyanidins (**I** and **II**) gave permethylates with an excess of methyliodide in the presence of NaH. These were hydrolyzed to form the same product, 2,3,4-tri-*O*-methyl-D-glucopyranose.

Thus, proanthocyanidin alhacidin had the structure and relative configuration 7-*O*-β-D-Glc*p*$\xrightarrow{6}$galloyl-(+)-catechin-[(4α-8)-(+)-catechin]3-(4α-8)-(–)-epigallocatechin-3-*O*-gallate.

EXPERIMENTAL

UV spectra of proanthocyanidins and their derivatives in EtOH were taken on a Perkin–Elmer Lambda-16 instrument; IR spectra, in KBr disks on a Perkin–Elmer System 2000 FTIR instrument. Optical activity was determined using a Zeiss polarimeter. ^{13}C NMR spectra of compounds in deuteroacetone and deuterated water mixtures were recorded on a Bruker AM 400/100 MHz instrument. Molecular weights were determined on a MOM 3170 ultracentrifuge using gel chromatography over a calibrated column of Sephadex LH-20. PC and TLC on Silufol UV-254 plates were used to identify compounds and determine their purity. We used the following solvent systems: *n*-BuOH:AcOH:H_2O 40:12:28 (1), $CHCl_3$:EtOAc 1:2-4 (2), *n*-BuOH:HCOOH (85%):H_2O 9.5:1:2 (3), HCl (2 N) (4), *n*-BuOH:Py:H_2O 6:4:3 (5). Detection used vanillin (1%) in alcoholic H_2SO_4 (5%); aqueous $FeCl_3$ and $K_3[Fe(CN)_6]$ solutions (1%) (1:1); and anilinium phthalate.

Extraction and Isolation of Proanthocyanidins. Ground air-dried aerial part of *A. pseudalhagi* (6.0 kg) was extracted with 80% EtOH (6 × 20 L). The resulting EtOH extracts were combined. The EtOH was vacuum distilled at 45–50°C. The

remaining thick extract (503.4 g, 8.9% of the air-dried raw material) was diluted with distilled water (1:1 v/v) and fractionated successively according to polarity of the organic solvents Et_2O (500 × 4), EtOAc (500 × 4), and *n*-BuOH (500 × 4).

The Et_2O, EtOAc, and *n*-BuOH extracts were evaporated to afford 43.5 g (0.72%), 35.2 (0.58), and 127.6 (2.12), respectively, of low-molecular-weight relatively slightly polar and polar total extracts. The remaining aqueous layer was evaporated in a porcelain dish on a water bath with constant stirring and dried. The resulting thick residue was ground to afford 297.1 g (4.95%) of total high-molecular-weight light-brown proanthocyanidins.

Separation of Proanthocyanidins. The *n*-BuOH extract (127 g) was mixed with cellulose (127 g), placed on a column of microcrystalline cellulose (140 × 6 cm, 1700 g), and eluted with $CHCl_3$, $CHCl_3$:EtOAc, EtOAc, EtOAc:$(CH_3)_2CO$, $(CH_3)_2CO$, and $(CH_3)_2CO$:H_2O. Fractions of 100–150 mL were collected. The elution was monitored using TLC. Homogeneous fractions (according to TLC) were combined and rechromatographed over a column of Sephadex LH-20 (140 × 3 cm, 158 g).

Proanthocyanidin I, 0.820 g, $C_{58}H_{52}O_{29}$, MW 1210–1220, $[\alpha]_D^{22}$ +132° (*c* 0.37, EtOH). UV spectrum (λ_{max}, nm): 225, 229, 253, 271; λ_{min} 200. IR spectrum (ν_{max}, cm^{-1}): 3589, 3388, 2936, 1719, 1609, 1513, 1450, 1271, 1165, 1077.

Table 1 lists the ^{13}C NMR spectrum.

Proanthocyanidin II, 0.875 g, $C_{95}H_{80}O_{45}$, MW 1935–1945, $[\alpha]_D^{22}$ +21.9° (*c* 0.28, EtOH). UV spectrum (λ_{max}, nm): 201, 222, 275; λ_{min} 200. IR spectrum (ν_{max}, cm^{-1}): 3387, 2936, 1702, 1606, 1510, 1450, 1365, 1242, 1076, 1042.

Table 2 lists the ^{13}C NMR spectrum.

Alkaline Cleavage of I. A 20-mL four-necked round-bottomed flask was charged with **I** (75 mg) and purged slowly with N_2. KOH solution (50%, 5 mL) was added. The mixture was constantly stirred. The lower part of the flask was immersed into a bath with a low-melting metallic alloy at 150–160°C that was heated over five minutes to 230°C. The mixture was rapidly cooled by immersing the flask into icewater acidified with H_2SO_4 (20%). The contents of the flask were diluted with water and extracted with EtOAc. The EtOAc extract was dried over anhydrous Na_2SO_4. The solvent was distilled off. The solid was chromatographed over a column of polyamide to afford three compounds with M^+ 126, mp 218–219°C, R_f 0.64 (PC, system 3) (phloroglucinol); M^+ 154, mp 200°C (dec.), R_f 0.72 (PC, system 3) (protocatechoic acid); and M^+ 170, mp 220–221°C, R_f 0.56 (PC, system 3) (gallic acid) [7, 18].

Alkaline cleavage of II (80 mg) was carried out by the method described for **I** to afford three compounds that were identified as phloroglucinol (**1**) and protocatechoic (**2**) and gallic acids (**3**).

Acid Cleavage of I. Compound **I** (90 mg) was dissolved in EtOH (4 mL), treated with HCl (1.5 mL, 2 N), and refluxed under N_2 on a water bath for 2 h. The mixture was diluted with water and extracted with EtOAc. The extract was washed with $NaHCO_3$ solution and dried over anhydrous Na_2SO_4. The solvent was distilled off. The solid was chromatographed over a column of Sephadex LH-20 with elution by EtOH (60%) to afford a compound (6 mg) of composition $C_{15}H_{14}O_7$, mp 215–216°C, $[\alpha]_D^{21}$ –56° (*c* 0.41, MeOH), R_f 0.42 (system 2) [(-)-epigallocatechin (**7**)] [7, 18].

Paper chromatography of the hydrolysate detected cyanidin (**6**), R_f 0.69 (system 4); glucose, R_f 0.51 (system 5); and a compound with mp 135–137°C, $[\alpha]_D^{24}$ –26.5° (*c* 0.21, acetone) [galloylglucose (**5**)].

Acid cleavage of II (85 mg) was carried out analogously to that of **I** to afford a compound (5 mg) of composition $C_{22}H_{18}O_{10}$, mp 210–211°C, $[\alpha]_D^{20}$ –135° (*c* 0.06, MeOH:H_2O, 1:1), R_f 0.6 (system 2) [(-)-epigallocatechin-3-*O*-gallate (**10**)]. Paper chromatography of the hydrolysate detected cyanidin (**6**), R_f 0.69 (system 4); glucose, R_f 0.51 (system 5); and a compound with mp 135–137°C, $[\alpha]_D^{24}$ –26.5° (*c* 0.21, acetone) [galloylglucose (**5**)].

Thiolytic Cleavage of Proanthocyanidins. Cleavage of I. Compound **I** (180 mg) and thiophenol (4 mL) were mixed, treated with HOAc (3 mL) in EtOH (10 mL), and left at room temperature for 48 h. The course of the reaction was monitored by TLC. The reaction mixture was condensed. The resulting oily residue was chromatographed over Sephadex LH-20 (elution by EtOH) to afford a compound (7 mg), $C_{15}H_{14}O_7$, mp 215–216°C, $[\alpha]_D^{21}$ –56° (*c* 0.41, MeOH), R_f 0.42 (system 2) [(-)-epigallocatechin (**7**)] and an amorphous substance (50 mg) of total thioethers [7, 18].

Cleavage of II. Compound **II** (190 mg) was cleaved and the reaction products were purified as described above. The reaction mixture was chromatographed over Sephadex LH-20 (60%) to afford a compound (15 mg) of composition $C_{22}H_{18}O_{10}$, mp 210–211°C, $[\alpha]_D^{20}$ –135° (*c* 0.06, MeOH:H_2O, 1:1), R_f 0.6 (system 1) [(–)-epigallocatechin-3-*O*-gallate (**10**)] and a thioether mixture (68 mg).

Cleavage of Thioethers I and II. Thioethers (68 mg) were mixed with an EtOH:HOAc mixture (3.5 mL, 9:1), treated with catalyst (Raney Ni), and held at 50°C for 1 h. The reaction mixture was filtered. The filtrate was condensed and chromatographed over Sephadex LH-20 (elution by 80% EtOH) to afford a compound of composition $C_{15}H_{14}O_6$, mp 178–179°C, $[\alpha]_D^{20}$ +18.6° (*c* 0.2, acetone:H_2O, 1:1), R_f 0.64 (system 1) [(+)-catechin (**9**)].

Methylation of I. A solution of **I** (80 mg) in DMSO (10 mL) was stirred, treated with NaH (0.10 g), held at room temperature for 1 h, treated dropwise with MeI (5 mL), stored for another 4 h, poured into icewater (30 mL), and extracted with $CHCl_3$. The extract was worked up with sodium thiosulfate, washed with water, and dried over anhydrous Na_2SO_4. The solvent was distilled off. The solid was methylated another five times. The final product was purified by chromatography to afford the amorphous permethylate (63 mg).

Hydrolysis of the Permethylate of I. The resulting permethylate (63 mg) was dissolved in aqueous MeOH (5 mL, 50%) containing H_2SO_4 (5%), heated for 8 h on a water bath, cooled, neutralized with $BaCO_3$, and filtered to remove the precipitate of $BaSO_4$. The filtrate was evaporated to dryness. The solid was purified by column chromatography to afford the methylated carbohydrate (28 mg) that was identified by comparison with an authentic sample of 2,3,4-tri-*O*-methyl-D-glucopyranose.

Methylation of II. The methylation was carried out analogously as above. The resulting permethylate (58 mg) was hydrolyzed to afford 2,3,4-tri-*O*-methyl-D-glucopyranose.

Enzymatic Hydrolysis of I and II. The glycoside (20 mg) was dissolved in H_2O (10 mL), treated with β-glucosidase, placed into a thermostat, and held at 30°C for 6 h. Polyphenols were precipitated by adding lead acetate solution. Paper chromatography of the filtrate detected glucose (R_f 0.51, system 5).

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