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CATECHINS AND PROANTHOCYANIDINS OF *Alhagi sparsifolia*. I*

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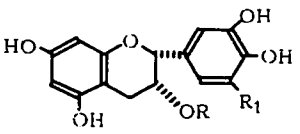
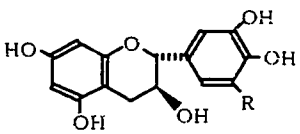
Ten individual compounds have been isolated from the epigeal part of Alhagi sparsifolia *Shap. in various stages of vegetative growth. Their structures have been established by a study of PMR spectra, physicochemical properties, and the products of chemical transformations: (−)-epicatechin, (−)-epigallocatechin, (−)-epigallocatechin gallate, (+)-catechin, (+)-gallocatechin, proanthocyanidin B-2, (−)-epigallocatechin-(4β-8)-(−)-epicatechin, epigallocatechin gallate-(4β-8)-(−)-epicatechin, proanthocyanidin B-1, and (−)-epicatechin-(4β-8)-gallocatechin.*

One of the widely distributed plants of Central Asia is camel thorn – *Alhagi* Gagneb., fam. Fabaceae Lindl. This genus includes four extremely widespread species: *A. khigirsorum, A. persarum, A. pseudoalhagi*, and *A. sparsifolia*. The last of these is the least studied in the chemical respect. Its roots and epigeal part have been found to contain tannins, anthocyans, coumarins, and flavonoids [1-3]. We have begun a study of the catechins and proanthocyanidins of *A. sparsifolia* Shap., a tincture and a decoction of which are used as cholegogics, diuretics, and sudorifics and in the treatment of dysentery and diseases of the stomach [4].

From an aqueous alcoholic extract of the epigeal part of the plant gathered in the phase of vigorous growth, flowering, and fruit formation in the Tashkent Oblast we have isolated by gel filtration on Sephadex LH-20, ten individual compounds, including five catechins (**1-5**) and five dimeric proanthocyanidins (**6-10**).





1. R=R$_1$=H
2. R=H; R$_1$=OH
3. R=Galloyl: R$_1$=OH

4. R=H
5. R=OH

Compounds (**1-5**) were identified as (−)-epicatechin (**1**), (−)-epigallocatechin (**2**), (−)-epigallocatechin 3-O-gallate (**3**), (+)-catechin (**4**), and (+)-gallocatechin (**5**) [5-11].

The structures of the dimeric proanthocyanidins isolated (**6-10**) have been established on the basis of the results of acid hydrolysis, alkaline and thiolytic cleavage, and spectral characteristics (UV, IR, PMR) and their comparison with the literature [12-20]. Compounds (**6**) and (**9**) have been identified as proanthocyanidins B-2 and B-1, respectively.

On thiolysis, compound (**7**) formed (−)-epicatechin and the thioether (**11**). Cleavage of the latter with Raney nickel led to (−)-epigallocatechin. On acid hydrolysis, the "upper" part yielded delphinidin (**12**), and the "lower" part (−)-epicatechin.

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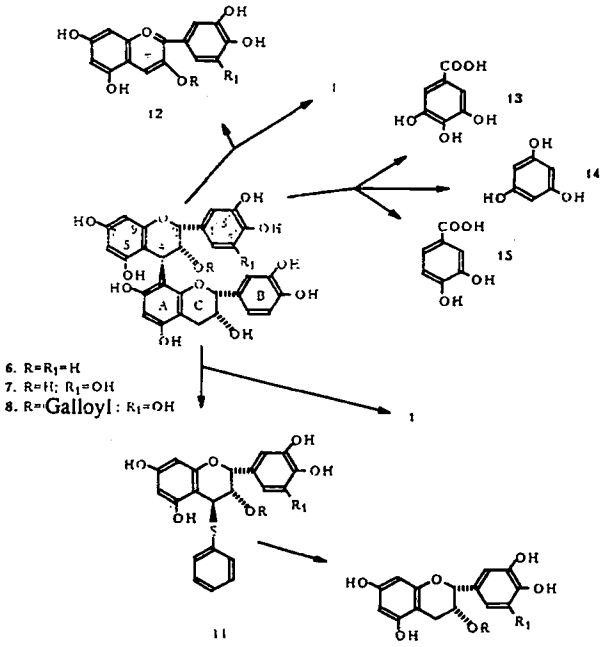
TABLE 1. CSs (δ, ppm) and SSCCs (J, Hz) of the Protons in the PMR Spectra of Proanthocyanidins (**6-10**)

Compound	H-2	H-3	H-4	H-2′	H-3′	H-4′	Ring *A*	Ring *B*	Galloyl
6	5.08 br.s	4.32 br.s	α4.72 br.s	4.93 br.s	3.98 br.s	2.73–2.91 m	5.95–6.08	6.71–7.13	
7	5.11 br.s	4.31 m	α4.74 br.s	4.97 br.s	4.01 m	2.76–2.86 m	5.93–6.11	6.69–7.20 6.78 s	
8	5.66 br.s	5.53 m	α4.81 d $J_{4,3}$=3	4.95 br.s	4.04 m	2.68–3.01 m	5.95–6.13	6.71–7.08 6.80c	6.98 s
9	5.10 br.s	4.15 m	α4.69 d $J_{4,3}$=2	4.79 d $J_{2,3}$=8	4.08 m	α2.80 dd $J_{4\alpha,3}$=6 $J_{4\alpha,4\beta}$=16 β2.56 dd $J_{4\beta,3}$=7 $J_{4\beta,4\alpha}$=16	5.92–6.09	6.54–6.98	
10	5.12 br.s	4.10 m	α4.71 d $J_{4,3}$=2	4.75 d $J_{2,3}$=8	4.06 m	α2.89 dd $J_{4\alpha,3}$=6 $J_{4\alpha,4\beta}$=16 β2.55 dd $J_{4\beta,3}$=8 $J_{4\beta,4\alpha}$=16	5.94–6.11	6.59–6.99 6.67 s	

Consequently, this proanthocyanidin was a mixed one, the "lower" block being (−)-epicatechin, and the "upper" one (−)-epigallocatechin.

The results of a study of the chemical shifts (CSs) and spin−spin coupling constants (SSCCs) of the signals in the PMR spectrum of compound (7) (Table 1) confirmed the information on structure obtained with the aid of chemical transformations. A two-proton singlet at 6.78 ppm related to the protons of ring *B* of (−)-epigallocatechin, while the protons of ring *B* of (−)-epicatechin appeared in the form of multiplets in the 6.69-7.20 ppm region. The signals of the protons of rings *C* of (−)-epigallocatechin and of (−)-epicatechin appeared, respectively, at 5.11 (1H, br.s, H-2), 4.31 (1H, m, H-3), 4.74 (1H, br.s, αH-4) and 4.97 (1H, br.s, H-2′), 4.01 (1H, m, H-3′), 2.76-2.86 ppm (2H, m, H-4′). The signals of the protons of rings *A* and *A′* H-6, H-8, and H-6′ appeared in the 5.93-6.11 ppm region. On the basis of the results obtained, it can be stated that proanthocyanidin (7) had the structure (−)-epigallocatechin-(4β-8)-(−)-epicatechin.

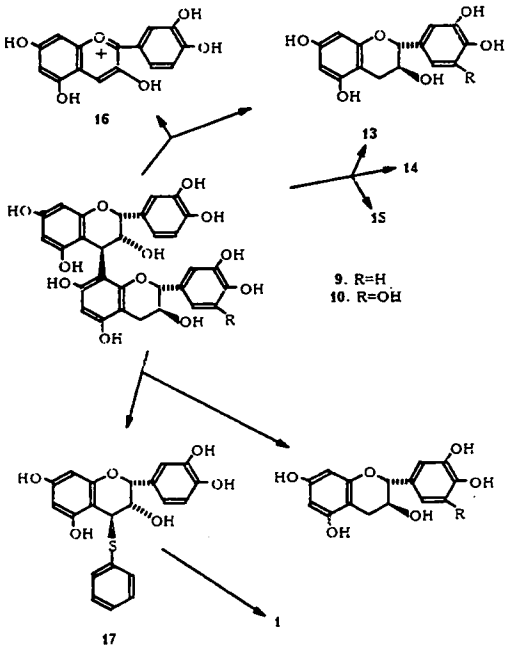


The physicochemical parameters and spectral characteristics of compound (**8**) were close to those of compound (**7**). However, in contrast to the latter, the PMR spectrum of (**8**) had, besides the characteristic signals of (−)-epicatechin and (−)-epigallocatechin, the characteristic signal of a gallic acid residue at 6.98 ppm (2H, s, H-2, H-6). Consequently, one of the blocks was galloylated. This was also shown by a paramagnetic shift of the signals of the H-3 proton of the "upper" block. The results of thiolytic cleavage followed by reduction of the thioether showed that it was in fact the "upper" − i.e., the (−)-epigallocatechin − block that was galloylated. Thus, it can be stated unambiguously that this dimeric anthocyanidin was [3-O-galloyl-(−)-epigallocatechin]-(4β-8)-(−)-epicatechin.

On acid hydrolysis, compound (**10**) gave cyanidin (**16**) and (+)-gallocatechin. The thiolytic cleavage of (**10**) gave (+)-gallocatechin and the thioether (**17**), the reductive degradation of which led to (−)-epicatechin. These results unambiguously showed that the proanthocyanidin consisted of "upper" epicatechin and "lower" gallocatechin.

The chemical shifts of the signals and the SSCCs in the PMR spectrum of (**10**) were as follows: the signal of the protons of ring *C* of the "upper" block appeared at 5.12 (1H, br.s, H-2), 4.10 (1H, m, H-3) and 4.71 (1H, d, $J_{4\alpha,3}$ = 2 Hz, αH-4) ppm, and those of ring *C* of the "lower" block at 4.75 (1H, d, $J_{2,3}$ = 8 Hz, H-2′), 4.06 (1H, m, H-3′), 2.89 (1H, dd, $J_{4\alpha,3}$ = 6 Hz, $J_{4\alpha,4\beta}$ = 16, αH-4′), 2.55 ppm, (1H, dd, $J_{4\beta,3}$ = 8 Hz, $J_{4\beta,4\alpha}$ = 16, βH-4′).

The signals of the three protons of rings *A* and *A′* − H-6, H-8, and H-6′ − appeared in the 5.94-6.11 ppm region. The signals of the epicatechin ring *B* appeared in the form of a multiplet in the 6.59-6.99 ppm region, while the protons of the gallocatechin ring *B′* resonated in the form of a singlet at 6.67 ppm (see Table 1).



On the basis of the results given above, it may be asserted that proanthocyanidin (**10**) had the structure and stereochemistry (−)-epicatechin-(4β-8)-(+)-gallocatechin.

EXPERIMENTAL

General Information. The UV spectra of the catechins and proanthocyanidins were obtained in alcoholic solution on a Hitachi EPS-3T instrument, IR spectra on a Carl Zeiss, Jena, instrument in tablets with potassium bromide, and NMR spectra on a Tesla BS-567A/100 MHz instrument in $(CD_3)_2CO$ and $(CD_3)_2CO-D_2O$ (1:1) solutions with HMDS as internal standard (δ-scale). Concentrations ranged from 10 to 20%.

To check the homogeneity of the substances we used PC and TLC on Silufol UV-254 plates, with the chromatographic systems; 1) 6% acetic acid (v/v); 2) BAW (14:1:5), v/v/v), and 3) 2 N hydrochloric acid.

Elementary analyses agreed with the calculated figures.

Extraction and Isolation of the Catechins and Proanthocyanidins. The epigeal part of the plant (8 kg) was extracted six times with 80% ethanol and the extract was evaporated in vacuum at 40°C to 2 liters. The concentrated extract was exhaustively treated successively with diethyl ether, ethyl acetate, and *n*-butanol, which gave, respectively, 42.1, 38.8, and 138.2 g of extractives. The aqueous residue from the above solvent treatment yielded 352 g of viscous extract.

Separation of the Catechins. The diethyl ether extract (42 g) was chromatographed on a column of Sephadex LH-20 (5 × 180 cm), with elution by the systems ethyl acetate–hexane (1:2-4:1) and ethanol. The following compounds were isolated and were identified by their physicochemical constants: (−)-epicatechin (**1**) − 0.249 g; (−)-epigallocatechin (**2**) − 0.341 g; (−)-epigallocatechin 3-O-gallate (**3**) − 0.102 g; (+)-catechin (**4**) − 0.021 g; and (+)-gallocatechin (**5**) − 0.060 g.

Separation of the Dimeric Proanthocyanidins. The ethyl acetate fraction (48.8 g) was chromatographed on a column of Sephadex LH-20 (5 × 180 cm), using ethanol for elution. Five dimeric proanthocyanidins were obtained.

Proanthocyanidin B-2 (6). Amorphous powder with the composition $C_{30}H_{26}O_{12}$, M 578, $[\alpha]_D^{23}$ +25.1° (*c* 1.0 acetone). R_f (1) 0.58, R_f (2) 0.42. IR spectrum: ν_{max} 3335 (OH), 1621 cm^{-1} (aromatic rings). For its PMR spectrum, see Table 1.

Proanthocyanidin B-1 (9). Amorphous powder (0.059 g) with the composition $C_{30}H_{26}O_{12}$, M 578, $[\alpha]_D^{23}$ +32.8° (*c* 0.41, acetone). R_f (1) 0.50, R_f (2) 0.31. IR spectrum: ν_{max} 3330 (OH), 1620 cm^{-1} (aromatic rings). For its PMR spectrum, see Table 1.

(−)-Epigallocatechin-(4β-8)-(−)-epicatechin (7). Amorphous powder (0.387 g) with the composition $C_{30}H_{26}O_{13}$, M 594, $[\alpha]_D^{23}$ +48.6° (*c* 1.1; acetone). R_f (1) 0.48, R_f (2) 0.29. IR spectrum: ν_{max} 3400, 1625, 1545, 1510, 1435, 1320, 1200, 1038, 831, 805, 770, 732 cm^{-1}.

(−)-Epigallocatechin 3-O-gallate-(4β-8)-(−)-epicatechin (8). Amorphous powder (0.307 g) with the composition $C_{37}H_{30}O_{18}$. M 762, $[\alpha]_D^{23}$ +69.8° (*c* 0.88; acetone). R_f (1) 0.56, R_f (2) 0.38. IR spectrum: ν_{max} 3500, 1695, 1615, 1450, 1320, 1250, 1040, 830, 805, 775, 740 cm^{-1}.

(−)-Epicatechin-(4β-8)-(+)-gallocatechin (10). Amorphous powder (0.213 g) with the composition $C_{30}H_{26}O_{13}$, M 594, $[\alpha]_D^{22}$ +22.5° (*c* 0.62; ethanol). R_f (1), 0.49, R_f (2) 0.25. IR spectrum: ν_{max} 3400, 1620, 1545, 1510, 1435, 1320, 1200, 1040, 830, 805, 774, 733 cm^{-1}.

Alkaline Cleavage of (7), (8), and (10). The cleavage of 50 mg of each substance was carried out by the procedure described in [18]. From the reaction media we isolated and identified phloroglucinol (**14**) and protocatechuic (**15**) and gallic (**13**) acids.

Acid Cleavage of (7), (8), and (10). The cleavage of 80 mg of each substance was carried out by the procedure described in [18]. As a result, (**7**) and (**8**) yielded (−)-epicatechin, $C_{15}H_{14}O_6$. M 290, mp. 241-243°C, $[\alpha]_D^{24}$ −69° (*c* 0.50; acetone–water, 1;1), while PC showed the presence in the hydrolysate of delphinidin, R_f (3) 0.36, λ_{max} 554 (0.1% HCl in ethanol).

Compound (**10**) yielded (+)-gallocatechin, $C_{15}H_{14}O_7$, M 306, mp 186-188°C, $[\alpha]_D^{24}$ +15° (*c* 0.10; ethanol) and cyanidin, R_f (3) 0.69, λ_{max} 252 (0.1% HCl in ethanol).

Thiolytic Cleavage of (7) and (8). A mixture of 100 mg of substance and 4 ml of phenyl mercaptan was treated with 2 ml of acetic acid in 10 ml of ethanol, and the reaction mixture was left at room temperature for 36 h. Then it was concentrated to an oily residue, which was chromatographed on Sephadex LH-20, with elution by ethanol. In both cases (−)-epicatechin and amorphous thioethers were obtained.

Cleavage of the Thioethers from (7) and (8). Each thioether was mixed with 3 ml of ethanol–acetic acid (9:1), the catalyst – Raney nickel – was added to the reaction mixture, and it was kept at 50°C for 1 h. Then it was filtered, and the filtrate was concentrated and chromatographed on Sephadex LH-20, with elution by ethanol. The first thioether yielded (−)-epigallocatechin $C_{15}H_{14}O_7$, M 306, mp 216-218°C, $[\alpha]_D^{24}$ −55° (*c* 0.33; methanol), and the second (−)-epigallocatechin 3-O-gallate $C_{22}H_{18}O_{11}$, M 458, mp 210-211°C, $[\alpha]_D^{24}$ −184.8° (*c* 0.69; water).

Thiolytic Cleavage of (10). The substance (100 mg) was cleaved by the method described above. This gave (+)-gallocatechin and a thioether. Reductive degradation of the thioether led to (−)-epicatechin.

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