Chemistry of Natural Compounds, Vol. 33, No. 4, 1997

PROANTHOCYANIDINS OF *Polygonum coriarium*
III. STRUCTURES OF PROANTHOCYANIDINS T1 AND T2*

B. M. Keneshov,[b] Z. A. Kuliev,[a] A. D. Vdovin,[a]
N. D. Abdullaev,[a] A. B. Makhmatkulov,[a]
and A. A. Nishanov[b]

UDC 547.984

The roots of Polygonum coriarum *have yielded two oligomeric proanthocyanidins, T1 and T2, and their structures have been established: 3-O-galloyl-7-O-[O-(6-O-galloyl)-β-D-glucopyranosyl]-(−)-epigallocatechin-(4β−8)-(−)-epicatechin-(4β−8)-(−)-epicatechin-(4β−8)-(−)-epigallocatechin 3-O-gallate (T1) and (−)-epicatechin-(4β−8)-[3-O-galloyl-(−)-epigallocatechin]-(4β−8)-(−)-epicatechin-(4β−8)-(+)-catechin (T2).*

A chemical study of *Polygonum coriarum* has been reported previously [1-5]. Continuing the study of the proanthocyanidins of the roots of *Polygonum coriarum* Grig., from a butanol-soluble fraction of an aqueous alcoholic extract we have isolated two oligomeric proanthocyanidins, T1 and T2. The chemical structures of these compounds have been deduced from the results of a study of their physical properties and spectral characteristics (UV, IR, ^{13}C NMR)) and an analysis of chemical transformations. Analysis of the UV and IR spectra (see the Experimental section) permitted compound T1 to be assigned to the oligomeric proanthocyanidins. To determine its monomeric composition and establish its structure we performed a number of chemical transformations. The alkaline cleavage of T1 in a nitrogen atmosphere formed three compounds, which were identified from their physicochemical characteristic as phloroglucinol (**1**), protocatechuic acid (**2**), and gallic acid (**3**). The acid hydrolysis of T1 led to the formation of molecules of (−)-epigallocatechin 3-O-gallate (**4**), cyanidin (**5**), delphinidin (**6**), and β-glucose acylated by gallic acid (**7**). By mild thiolytic cleavage, from the "lower" block we obtained (−)-epigallocatechin 3-O-gallate (**4**), and from the "upper" block a mixture of two thioethers (**8** and **9**), which were subjected to catalytic degradation in the presence of Raney nickel. The substances obtained were identified as (−)-epicatechin (**10**) and (−)-epigallocatechin 3-O-gallate (**4**).

Thus, the results of the chemical investigations showed that proanthocyanidin T1 was an acylglycosylated oligomeric proanthocyanidin consisting of (−)-epicatechin and (−)-epigallocatechin 3-O-gallate.

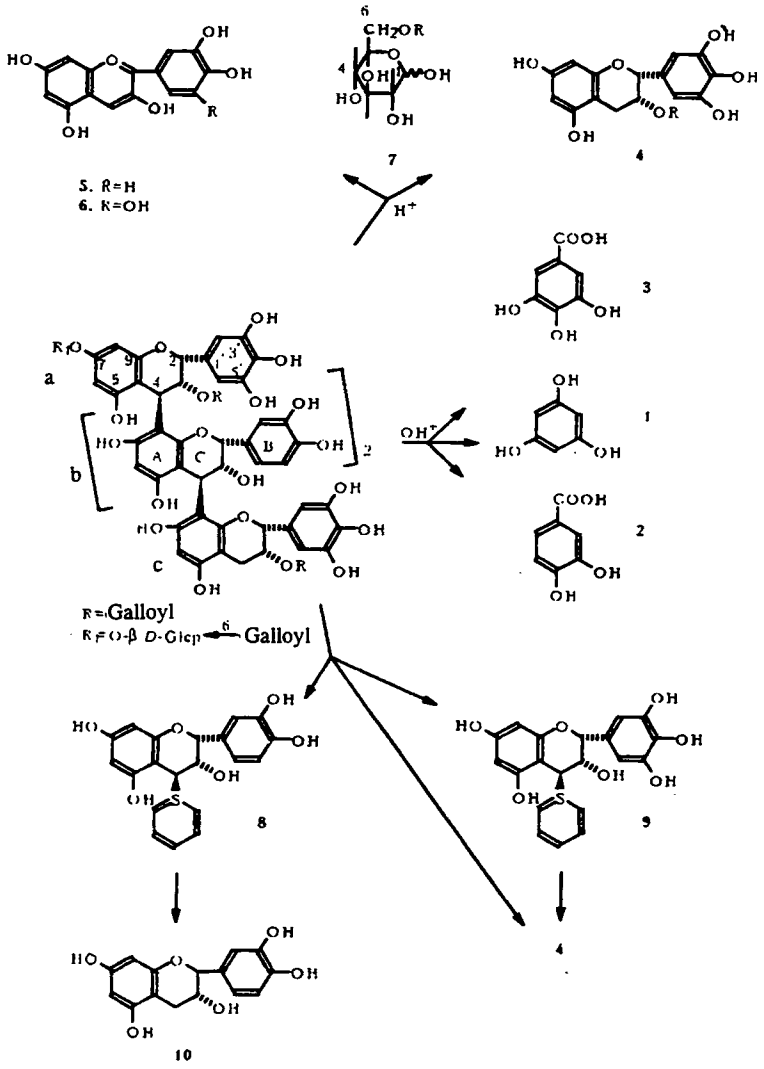
In actual fact, in the ^{13}C NMR spectrum of T1 (Table 1) obtained under conditions of complete suppression of spin-spin coupling with protons, signals characteristic for epigallocatechin, epicatechin, β-glucose, and gallic acid were detected [6-8]. A broad resonance signal in the 153.5-155.5 ppm region related to the C-5, C-7, and C-9 carbon atoms, and a signal at 96.0 belonged to C-6 and the unsubstituted C-8 atom of a phloroglucinol nucleus. Resonance signals at (ppm) 109.6 (C-2′ and C-6′), 145.0 (C-3′ and C-5′), and 132.8 (C-4′) showed the presence of a gallocatechin block, while signals at 114.2, 115.0, and 118.4 ppm, relating to the C-2′, C-5′, and C-6′ carbon atoms, respectively, showed that there was a catechin block in T1. The absence of signals at about 81-83 ppm (C-2) showed that proanthocyanidin T1 consisted only of blocks with the 2,3-*cis*-configuration. The resonance in the regions of 75.5 and 34.1 ppm and 77.9, 69.1, and 25.4 ppm of signals relating to the C-2, C-3, and C-4 atoms of the heterocycle showed that one of the "upper" and "lower" blocks was galloylated in the C-3 position. Signals in the region of chemical shifts at 100.4-102.7 ppm, relating to the C-10 atoms, were characteristic for proanthocyanidins with C-4−C-8 interflavan bonds.

*The material of this paper were presented at the IInd International Symposium on the Chemistry of Natural Compounds (SCNC, Eskişehir, Turkey, October 22-24, 1996).

a) Institute of the Chemistry of Plant Substances, Academy of Sciences of the Republic of Uzbekistan, Tashkent, fax (3712) 40 64 75; *b*) Osh State University. Translated from Khimiya Prirodnykh Soedinenii, No. 4, pp. 588-593, July-August, 1997. Original article submitted February 24, 1997.

0009-3130/97/3304-0453$18.00

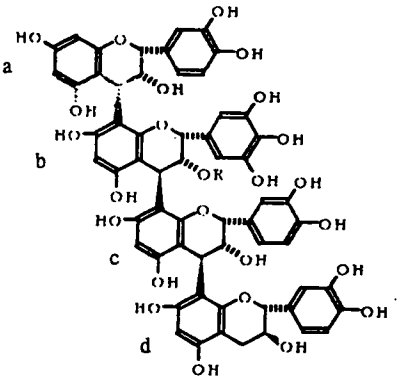
Signals of the C-1, C-3, and C-5 atoms of glucose at 102.7, 77.9, and 75.5 ppm, respectively, showed that the anomeric center had the β-configuration [9]. That the carbohydrate residue was acylated in the sixth position was shown by the presence of a signal at 65.4 ppm relating to a substituted C-6 atom of glucose.



Enzymatic cleavage in the presence of the enzyme β-glucosidase and acid hydrolysis of the permethylate of T1 showed that the carbohydrate residue consisted of a glucose molecule acylated by gallic acid in the sixth position and linked to the aglycon by a β-glycosidic bond.

The results of mild thiolytic cleavage of T1 indicated the absence of a sugar residue in the "lower" block of the molecule. In view of this, and also of the stereochemical hindrance in the "middle" blocks of a proanthocyanidin, we suggest the C-7 position of the "upper" block as the most probable position of attachment of the acylated glucose.

From its physicochemical properties and spectral parameters (UV, IR, and ^{13}C NMR), proanthocyanidin T2 was assigned to the mixed type of proanthocyanidins. Analysis of the chemical shifts of the carbon atoms in the ^{13}C NMR spectra showed that T2 consisted of molecules of (−)-epicatechin, (−)-epigallocatechin, and (+)-catechin and a gallic acid residue (see Table 2). The signals belonging to the C-2 and C-3 carbon atoms of the heterocycle appeared at 75.5 ppm and that of C-4 at 31.0 ppm, from which it followed that one of the "upper" blocks was galloylated. The lower block in proanthocyanidin T2 had the 2,3-*trans*-configuration and was not galloylated, as was shown by the CSs of the signals of the C-2, C-3, and C-4 carbon atoms (81.5, 65.2, and 27.7 ppm, respectively) [10-13]. The position of the interflavan bond in T2 was established on the basis of the same facts as for T1.



T2. R=Galloyl

The results of a study of the chemical transformations of T2 obtained by acid hydrolysis (compounds (**5**) and (**6**) and catechin) and alkaline cleavage (compounds (**1**), (**2**), and (**3**)) and thiolytic cleavage (substances (**4**) and (**10**) and catechin) confirmed the facts given above.

EXPERIMENTAL

General Information. The UV spectra of the proanthocyanidins and their derivatives were taken in alcoholic solution on a Hitachi EPS-3T instrument, and IR spectra on a Carl Zeiss, Jena, UR-20 in tablets with potassium bromide. ^{13}C NMR spectra were obtained on a Tesla BS 567 A/25 MHz instrument in Me_2CO-d_6 — D_2O (1:1) solution with TMS as internal standard, δ-scale. The concentration of the substances was about 20%. Molecular masses were determined on a MOM-3170 ultracentrifuge and by gel chromatography on a calibrated column of Sephadex LH-20. To check the homogeneity of the substances we used PC and TLC on Silufol UV-254 plates [4]. The elemental analyses of all the compounds corresponded to the calculated figures.

Isolation of the Total Proanthocyanidins. The ground roots of *Polygonum coriarium* (5.3 kg) were extracted six times with 80% ethanol. The extracts obtained were combined and were evaporated in vacuum at 50°C. The viscous extract was diluted with water (1:3) and was exhaustively extracted successively with diethyl ether, ethyl acetate, and *n*-butanol, giving, respectively, 12.7, 17.3, and 256.5 g of the corresponding fractions. The aqueous residue after evaporation of the solvent yielded a total of 1480 g of light brown substances.

Separation of the Proanthocyanidins. A mixture of the butanolic extract (50 g) and cellulose (50 g) was transferred to a column of microcrystalline cellulose (6 × 180 cm, 1400 g) and eluted with chloroform–ethyl acetate (1:10-1:20), ethyl acetate, and ethyl acetate–acetone (20:1-1:15) with the collection of 100-ml fractions. Similar fractions were combined. The eluates containing homogeneous substances were rechromatographed on a column of Sephadex LH-20 (5 × 160 cm) with elution by water–ethanol (2:3–1:4) and the collection of fractions with a volume of 10-15 ml. The homogeneity of the fractions was checked by TLC.

Proanthocyanidin T1 (0.648 g) had the composition $C_{82}H_{66}O_{43}$, M 1738, $[\alpha]_D^{28}$ +89°(*c* 0.9; acetone–water (2:1)). UV spectrum: λ_{max} 220, 245, 278, 310 nm; λ_{min} 258 nm. IR spectrum: ν_{max} 3400, 1695, 1620, 1545, 1460, 1340, 1250, 1110, 1040, 860, 830, 805, 770, 745 cm^{-1}. For the ^{13}C NMR spectrum, see Table 1.

Proanthocyanidin T2 (0.901 g) had the composition $C_{67}H_{54}O_{29}$, M 1322, $[\alpha]^{28}$ +77.6° (*c* 1.1; acetone–water (2:1)). UV spectrum: λ_{max} 220, 243, 280, 305 nm; λ_{min} 258 nm. IR spectrum: ν_{max} 3400, 1690, 1615, 1545, 1450, 1320, 1250, 1120, 1040, 830, 807, 774, 740 cm^{-1}. For the ^{13}C NMR spectrum, see Table 2.

Alkaline Cleavage of T1 and T2. The cleavage of 0.05 g of each substance was carried out by the procedure described in [4, 14]. As a result, we detected and identified phloroglucinol (**1**), protocatechuic acid (**2**), and gallic acid (**3**).

Acid Cleavage of T1 and T2. Each substance (0.15 g) was cleaved by the procedure described in [4, 14]. On the cleavage of T1 the following compounds were obtained and identified: (–)-epigallocatechin 3-O-gallate (**4**), $C_{22}H_{18}O_{11}$, mp 211-212°C, $[\alpha]^{22}$ –175° (*c* 0.15; methanol), cyanidin (**5**), delphinidin (**6**), and glucose 6-O-gallate (**7**) $C_{13}H_{16}O_{10}$, mp 137-138°C, $[\alpha]^{23}$ +22°, (*c* 0.32; acetone), and on the cleavage of T2: (+)-catechin, $C_{15}H_{14}O_6$, mp 178-180°C, $[\alpha]^{22}$ +21 (*c* 0.51; acetone–water (1:1)).

TABLE 1. Chemical Shifts (ppm) of the Signals of Carbon Atoms in the ^{13}C NMR Spectrum of Proanthocyanidin T1

Carbon atom	Fragment of T1				
	a	b	c	galloyl	glucose
C-2	75.5	77.9	77.9		
C-3	75.5	72.5	69.1		
C-4	34.1	35.9; 36.5*	25.4		
C-6	96.0	96.0	96.0		
C-8	96.0	106.1	106.1		
C-10	102.7[s]	100.4[s]	100.4[s]		
C-5, 7, 9			153.5–155.5*		
C-1′	131.3	131.3	131.3	120.6	102.7[s]
C-2′	109.6	114.2	109.6	109.6	75.5
C-3′	145.0[r]	145.0[r]	145.0[r]	145.0[r]	77.9
C-4′	132.8	143.8	133.3	138.9	70.8
C-5′	145.0[r]	115.0	145.0[r]	145.0[r]	75.5
C-6′	109.6	118.4	109.6	109.6	65.4
–COO–				164.5	
				166.4	

Signals marked with the same superscript letters may be interchanged. Underlining indicates galloylation of the hydroxy group at C-3.
*The chemical shifts of the carbon atoms in the two *b* fragments are not equivalent.

TABLE 2. Chemical Shifts (ppm) of the Signals of the Carbon Atoms in the ^{13}C NMR Spectrum of Proanthocyanidin T2

Carbon atom	Fragment of T2				
	a	b	c	d	galloyl
C-2	77.5[i]	75.5	76.8[i]	81.5	
C-3	70.8[j]	75.5	70.2[j]	65.2	
C-4	36.6	31.0	36.6	27.7	
C-6	95.7	106.5	106.5	106.5	
C-8	95.7	95.7	95.7	95.7	
C-10	100.1[s]	100.1[s]	103.1[s]	100.1[s]	
C-5,7,9	153.7	153.7	153.7	153.7	
C-1′	130.8	130.8	130.8	130.8	121.7
C-2′	114.2	109.7	114.2	114.2	109.7
C-3′	144.8	144.8	144.8	144.8	144.8
C-4′	144.8	134.6	144.8	144.8	139.1
C-5′	115.5	144.8	115.5	115.5	144.8
C-6′	118.9[k]	109.7	119.9[k]	118.9[k]	109.7
–COO–					164.4

Signals marked with the same superscript letters may be interchanged. Underlining indicates galloylation of the hydroxy group at C-3.

Thiolytic Cleavage of T1. A mixture of 312 mg of T1 and 4 ml of thiophenol was treated with 2 ml of acetic acid and 10 ml of ethanol and was left at room temperature for 48 h. The reaction mixture was concentrated to an oily residue, which was chromatographed on Sephadex-LH-20 (1 × 160 cm) with elution by ethanol. This gave 11 mg of (−)-epigallocatechin 3-O-gallate (**4**) and 229 mg of an amorphous substance — a mixture of the two thioethers (**8**) and (**9**).

Cleavage of the Thioethers (8) and (9). The thioethers (229 mg) were mixed with 4 ml of ethanol–acetic acid (9:1). Raney nickel catalyst was added to the reaction mixture and it was kept at 50°C for 3 h and was then filtered, and the filtrate was concentrated and chromatographed on a column of Sephadex LH-20 with elution by 80% ethanol. Two compounds were obtained: 9 mg of (−)-epigallocatechin-3-O-gallate and 11 mg of (−)-epicatechin $C_{15}H_{14}O_6$, mp 242-243°C, $[\alpha]_D^{22}$ −71° (*c* 0.11; acetone–water (1:1)).

Thiolytic Cleavage of T2. Proanthocyanidin T2 (433 mg) was cleaved and the reaction products were purified as described above. This yielded 22 mg of (+)-catechin and 297 mg of a mixture of thioethers. Catalytic cleavage of the thioethers and purification of the substances obtained led to 38 mg of (−)-epicatechin and 15 mg of (−)-epigallocatechin 3-O-gallate.

Methylation of T1. Methylation and the detection of the methylated sugars were carried out by the procedure described in [4]. This gave 2,3,4-tri-O-methyl-*D*-glucopyranose. The TLC of a hydrolysate of the permethylate likewise showed the presence of a methylated sugar (chloroform–methanol (12:1) system).

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