Chemistry of Natural Compounds, Vol. 33, No. 5, 1997

PROANTHOCYANIDINS OF *Polygonum coriarium*. IV. STRUCTURES OF PROANTHOCYANIDINS T3 AND T4*

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UDC 547.984

Two oligomeric proanthocyanidins have been isolated from the roots of Polygonum coriarium. *By a study of their physical properties and spectral characteristics and analysis of the results of chemical transformations, the chemical structures of these compounds have been established as: (−)-epicatechin-7-[O-β-D-glucopyranosyl]$_3$←^{6}—O-β-D-glucopyranosyl←^{6}—(m-trigalloyl)-[(4β-6)-(−)-epigallocatechin]$_2$-(4β−6)-(−)-epigallocatechin—T3; and (−)-epicatechin-3-O-galloyl-7-[O-β-D-glucopyranosyl]$_3$← O-β-D-glucopyranosyl←^{6}—galloyl-[(4β-6)-(−)-epicatechin]$_4$-(4β-6)-(−)-epigallocatechin — T4.*

Continuing a study of the chemical composition of the roots of *Polygonum coriarium* Grig., from a butanolic extract we have isolated two oligomeric proanthocyanidins: T3 and T4. The chemical study and isolation of a number of catechins and proanthocyanidins from this plant have been reported previously [1-6]. From the results of chemical transformations and physicochemical and spectral characteristics (UV, IR, ^{13}C NMR) the oligomeric proanthocyanidins isolated have been assigned to the acylglycosylated proanthocyanidins.

The alkaline fusion of T3 under conditions excluding oxidation of the cleavage products in an atmosphere of nitrogen led to the formation of phloroglucinol (**1**) and gallic and protocatechuic acids (**2** and **3**). The cleavage of T3 with a 5% solution of concentrated hydrochloric acid in butanol in a sealed tube gave cyanin (**4**), delphinidin (**5**), glucose (**6**), and gallic acid. These results showed the mixed nature of the proanthocyanidin, and this was confirmed by a study of the products of thiolytic cleavage (Scheme 1).

To determine its relative configuration and chemical structure, we studied the ^{13}C NMR spectrum of T3 obtained under conditions of complete suppression of spin-spin coupling with protons. Table 1 gives the results of our assignment of the chemical shifts (CSs) of the resonance signals of the carbon atoms in the ^{13}C NMR spectra. A consideration of the CSs of the carbon atoms of ring *B* in this compound permitted the identification of epicatechin and epigallocatechin systems in it. An intense signal at 144.9 ppm was assigned to the C-3′ and C-4′ atoms of ring *B* of epicatechin and also the C-3′ and C-5′ atoms of ring *B* of epigallocatechin.

The C-4′ carbon atom of epigallocatechin resonated at 134.8 ppm. A signal at 130.1 ppm was assigned to the C-1′ atoms of rings *B* of the catechins. An intense signal at 109.1 ppm was given by the C-2′ and C-6′ atoms of ring *B* of epigallocatechin. Resonance signals at 115.0, 115.3, and 120.0 ppm were assigned to the unsubstituted carbon atoms of ring *B* of epicatechin. Signals in the 154.6-155.7 region were assigned to the C-5, C-7, and C-9 atoms of the phloroglucinol nucleus — ring *A* — and an intense resonance signal at 106.6 ppm to the substituted C-6 atom of the same ring. Signals at 98.6 and 95.3 ppm related, respectively, to C-8 and C-6 atoms free from interflavan bonds, and one at 99.1 ppm to the C-10 atoms of rings *A* of the proanthocyanidin blocks. The chemical shifts of the C-10 atoms in proanthocyanidin T-3 were characteristic for proanthocyanidins with a C-4-C-6 interflavan bond [7].

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

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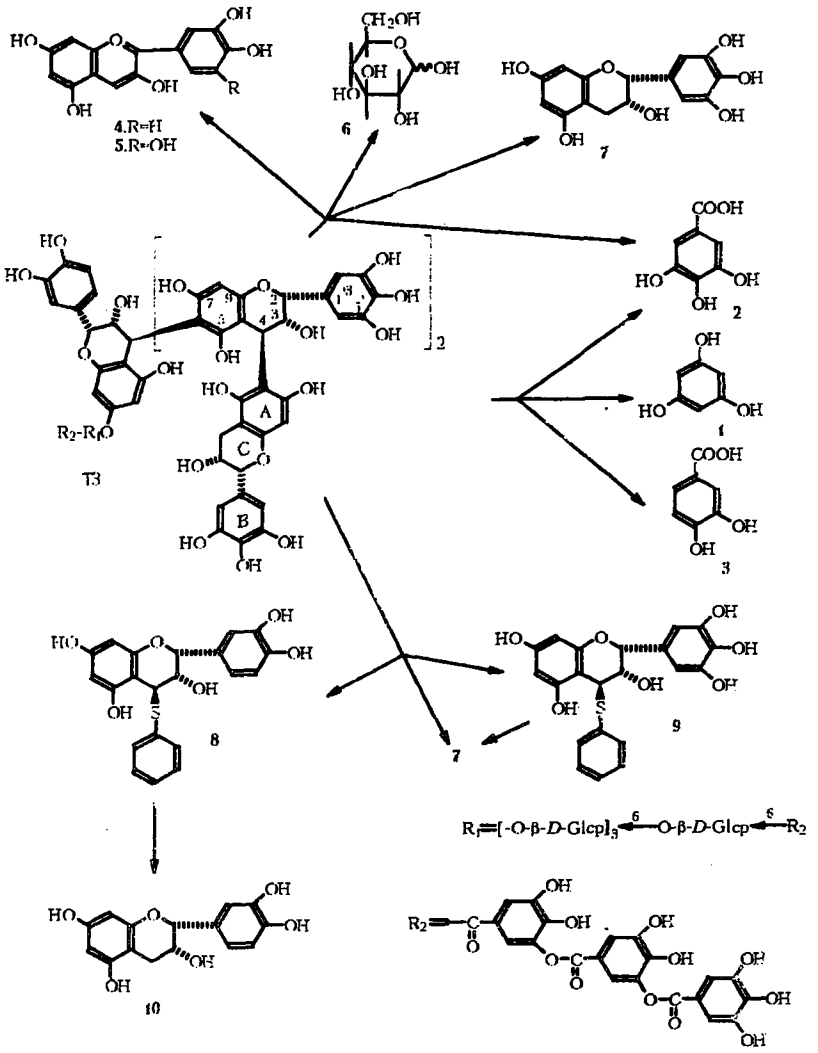
0009-3130/97/3305-0548$18.00

Scheme 1



The signals of the C-2 atoms of heterocycles *C* of catechins appeared at 77.6 and 79.3 ppm, which showed the absence of 2,3-*trans*-substituted blocks. The C-3 atoms in the same rings resonated at 71.5, 70.5, and 65.3 ppm. The substituted C-4 atoms gave signals at 35.4-36.7 ppm, while the signal of the unsubstituted C-4 atom of the "lower" block was masked by a signal of the solvent. Analysis of the spectra of the proanthocyanidins in the light of the influence of a substituent at C-4 and the effects of the galloylation of a hydroxy group at C-3 of the heterocycle permitted an assignment of the signals of all the C-2, C-3, and C-4 atoms, the establishment of the stereochemistries of the substituents of rings *C* of the catechin residues, and a determination of the fact that in not one of the blocks composing proanthocyanidin T3 was the hydroxy group at C-3 galloylated [8].

The ^{13}C NMR spectrum of T3 contained signals from three gallic acid residues: the signals of carbonyl carbons at 163.6, 165.3, and 166.8 ppm, and the signals of the C-1′ carbon atoms at 120.0 and 129.1 ppm, C-2′ at 110.0 ppm, and C-3′ at 144.9 ppm. In this case the resonance signals of the C-4′ and C-6′ atoms had undergone diamagnetic shifts (132.7 and 109.1 ppm) and that of C-5′ a paramagnetic shift (151.0 ppm) in comparison with the corresponding resonance signals of gallic acid (138.2, 110.0, and 144.9 ppm). Analysis of these dia- and paramagnetic effects showed that the gallic acid residues were linked to one another in the *meta*- position (C-5′) and formed *m*-trigallic acid.

In addition to these, the ^{13}C NMR spectrum of proanthocyanidin T3 included signals from the carbon atoms of sugar residues. A study of this group of signals provided the possibility of assigning them to the carbon atoms of four glucose residues linked in succession by 6-1 bonds. The signals of the carbon atoms of the anomeric centers appeared at 103.2 ppm, those of the C-2 and C-5 atoms at 75.5 ppm, those of C-3 atoms at 77.6 ppm, and those of the C-4 atoms at 70.6 ppm. The substituted C-6 atoms of the first, second, and third glucose residues resonated at 65.3 ppm, while the C-6 atom of the terminal glucose had a CS of 63.5 ppm. The latter is characteristic for glucose acylated by gallic acid in the sixth position [9-11]. Con-

TABLE 1. Chemical Shifts (ppm) of the Signals of the Carbon Atoms in the ^{13}C NMR Spectrum of Proanthocyanidin T3

Carbon atom	T3 fragment							
	a	b	c	$(Glcp)_3$	Glcp	Galloyl		
C-2	77.6	77.6	79.3					
C-3	71.5	70.5	65.3					
C-4	36.7	35.4	-					
C-6	95.3	106.6	106.6					
C-8	95.3	95.3	95.3					
C-10	103.2	99.1[s]	98.6[s]					
C-5,7,9	154.6; 155.7							
C-1′	130.1[y]	130.1[y]	130.1[y]	103.2	103.2	120.0	129.1[y]	129.1[y]
C-2′	115.0[x]	109.1[z]	109.1[z]	75.5	75.5	110.0[x]	110.0[x]	110.0[x]
C-3′	144.9	144.9	144.9	77.6	77.6	144.9	144.9	144.9
C-4′	144.9	134.8	134.8	70.6	70.6	132.7	132.7	138.2
C-5′	115.3[w]	144.9	144.9	75.5	75.5	151.0	151.0	144.9
C-6′	120.0	109.1[z]	109.1[z]	65.3	63.5	109.1[z]	109.1[z]	110.0[x]
-COO-						166.8[r]	165.3[r]	163.6[r]

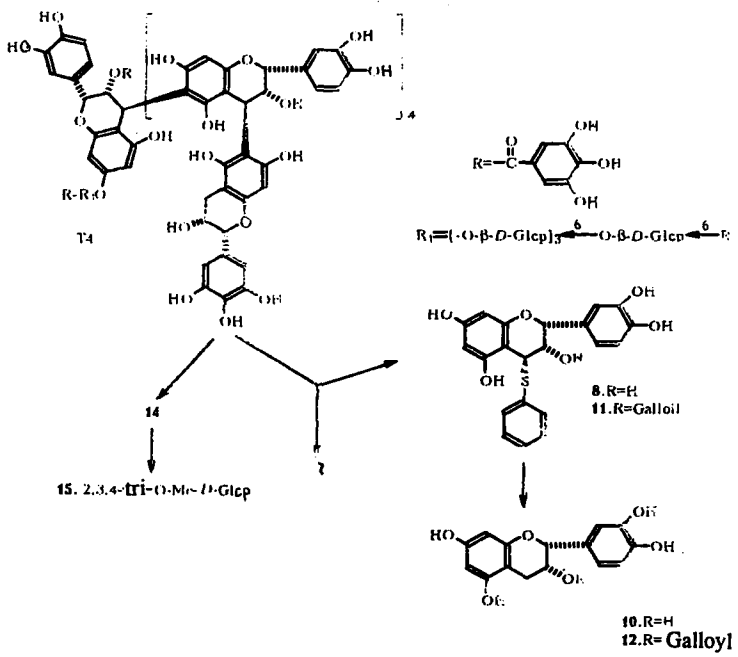
*Signals marked with the same superscript letters may be interchanged. Underlining denotes galloylation of the hydroxy group.

sequently, the glucan is a chain of four successively bound glucose residues containing a *m*-trigalloyl group in the terminal position. Exhaustive methylation of T3 followed by a study of the permethylate (**13**) confirmed the above-mentioned deductions. From the results of thiolysis it is most likely that the acylcarbohydrate part is attached to a C-7 atom of the "upper" block of the proanthocyanidin.

On the basis of what has been said above, for proanthocyanidin T3 we propose the most probable structure and configuration shown in Scheme 1.

On alkaline fusion, compound T4, like T3, formed (**1**), (**2**), and (**3**), and, on acid hydrolysis, it gave (**2**), (**4**), (**6**), and (**7**). Thiolytic cleavage led to substance (**7**) and a mixture of two thioethers, (**8**) and (**11**). After catalytic reduction of the thioethers in the presence of Raney nickel catalyst, compound (**10**) and epicatechin 3-O-gallate (**12**) were obtained. The carbohydrate moiety of T4 was analyzed by methylation and the hydrolysis of the permethylate (**14**) in an acid medium. This gave 2,3,4-tri-O-methyl-*D*-glucopyranose (**15**) (Scheme 2).

Scheme 2



Thus, the results of a chemical study showed that T4 is an oligomeric glycosylated proanthocyanidin. A comparison of the UV, IR, and ^{13}C NMR spectra of T3 and T4 revealed a close similarity of the corresponding spectra of these compounds. An interpretation of the chemical shifts of the signals of the carbon atoms in the ^{13}C NMR spectrum of T4 is given in Table 2. The presence in the T4 spectrum of signals close in characteristics but broadened in comparison with those of T3 indicated a higher molecular mass. The signals of the unsubstituted C-6 and C-8 carbon atoms appeared at 95.7 ppm. A signal at 106.3 ppm related to the C-6 atoms through which the interflavan bond is realized. The position of linkage of the flavan blocks through C-6 was shown by the chemical shift of the C-10 atoms (99.0 ppm). The presence of catechin blocks could be concluded from the presence of the corresponding C-2′, C-5′, and C-6′ signals (114.5, 115.5, and 118.0 ppm), and the presence of a gallocatechin system from the CS of the C-4′ signal (133.0 ppm). In proanthocyanidin T4, unlike T3, one of the "upper" epicatechin blocks is galloylated, as is shown by upfield shifts of the signals of the C-2 and C-4 atoms (75.3 and 32.4 ppm) and a downfield shift of the C-3 signal (73.9 ppm), and also by the presence of the signals of gallic acid residues in the ^{13}C NMR spectrum (see Table 2). A glucan consisting of four *D*-glucose residues contains one gallic acid residue in the terminal position. This was shown by the presence of resonance signals at 65.4 and 63.3 ppm corresponding to the C-6 atoms of glucopyranose residues.

The presence of four sugar residues, their sequence, the nature of their linkage with one another, and the position of their localization in the proanthocyanidin were established on the same grounds as in the study of T3. The results of chemical and spectral methods of investigation led to the conclusion that proanthocyanidin T4 has the structure and configuration shown in Scheme 2.

EXPERIMENTAL

General Information. The UV spectra of the proanthocyanidins and their derivatives were taken in alcoholic solution on a Hitachi EPS-3T instrument, and the IR spectra on a UR-20 (Carl Zeiss, Jena) 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 concentrations of the substances were about 20%. Molecular masses were determined on a MOM 3170 ultracentrifuge and by gel filtration 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 [5]. The elementary analyses of all the compounds corresponded to the calculated values.

Isolation of the Total Proanthocyanidins. The ground roots of *Polygonum coriarium* (5.3 kg) were extracted six times with 80% ethanol. The resulting extracts were combined and evaporated in vacuum at 50°C. The viscous extract was diluted with water (1:3) and was treated exhaustively with diethyl ether, ethyl acetate, and *n*-butanol. Fractions weighing 12.7, 17.3, and 256.5 g, respectively, were obtained.

Separation of the Proanthocyanidins. The butanolic extract was mixed with cellulose (50 g), transferred to a column of microcrystalline cellulose (6 × 180 cm, 1400 g) and eluted with chloroform–ethyl acetate, ethyl acetate, and mixtures of ethyl acetate and chloroform (20:1-1:15), with the collection of 100-ml fractions. Similar fractions were combined. The eluates that contained 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 10- to 15-ml fractions. The homogeneity of the fractions was monitored by TLC.

Proanthocyanidin T3 (0.709 g) had the composition $C_{105}H_{102}O_{59}$, M 2300, $[\alpha]_D^{26}$ +98.5° (*c* 0.57; acetone–water (1:1)). UV spectrum (nm): λ_{max} 220, 245, 280, 310; λ_{min} 260. IR spectrum (cm^{-1}): ν_{max} 3500, 1697, 1618, 1545, 1455, 1340, 1250, 1110, 1038, 860, 830, 805, 775, 743. For the ^{13}C NMR spectrum, see Table 1.

Proanthocyanidin T4 (1.085 g) had the composition $C_{128}H_{122}O_{65}$, M 2700, $[\alpha]_D^{26}$ +83.3°C (*c* 0.64; acetone–water (1:1)). UV spectrum (nm): λ_{max} 220, 245 278, 305; λ_{min} 259. IR spectrum (cm^{-1}): ν_{max} 3500, 1695, 1620, 1545, 1455, 1340, 1250, 1110, 1040, 860, 830, 805, 775, 740. For the ^{13}C NMR spectrum, see Table 2.

Alkaline Cleavage of T3 and T4. The cleavage of each substance (0.08 g) was carried out by the procedure described in [5, 12]. As a result, phloroglucinol and protocatechuic and gallic acids were detected and identified.

Acid Cleavage of T3 and T4. Each substance (0.15 g) was cleaved by the procedure described in [5, 12]. The cleavage of T3 gave (–)-epigallocatechin (7), mp 215-216°C, $[\alpha]_D^{23}$ –58°C (*c* 0.9, methanol), λ_{max} 272 (log ε 3.10), and in the hydrolysate, by PC, we detected cyanidin, R_f 0.8 (2 N HCl), λ_{max} 518 mμ (0.1% HCl in ethanol); delphinidin, R_f 0.36 (2 N

TABLE 2. Chemical Shifts (ppm) of the Signals of Carbon Atoms in the ^{13}C NMR Spectrum of Proanthocyanidin T4

Carbon atom	T4 fragment					
	a	b	c	$(Glcp)_2$	Glcp	Galloyl
C-2	75.3	76.6	79.0			
C-3	73.9	71.1	65.4			
C-4	32.4	36.4	–			
C-6	95.7	106.3	106.3			
C-8	95.7	95.7	95.7			
C-10	102.9	99.0	99.0			
C-5,7,9	154.6; 156.0					
C-1′	130.0[r]	130.0[r]	131.8[r]	102.9	102.9	120.1
C-2′	114.5	114.5	109.6	73.9	73.9	109.6
C-3′	144.7	144.7	144.7	76.6	76.6	144.7
C-4′	144.7	144.7	133.0	69.2	69.2	138.2
C-5′	115.5	115.5	144.7	75.3	75.3	144.7
C-6′	118.0	118.0	109.6	65.4	63.3	109.6
-COO-						164.4
						166.7

*Signals marked with the same superscript letters may be interchanged. Underlining denotes galloylation of the hydroxy group.

HCl), λ_{max} 554 mμ (0.1% HCl in ethanol); *D*-glucose, R_f 0.51 (*n*-butanol–pyridine–water (6:4:3) system); and gallic acid. After the cleavage of T4 we detected (–)-epigallocatechin, cyanidin, *D*-glucose, and gallic acid.

Thiolytic Cleavage of T3. A mixture of 0.305 g of the substance and 4 ml of phenyl mercaptan was treated with 2 ml of acetic acid in 10 ml of ethanol, and the resulting reaction mixture was left at room temperature for 36 h. During the first 8 h the course of the reaction was monitored by TLC every hour, and the mixture became thicker. An oily residue was obtained which was chromatographed on Sephadex LH-20 with elution by 80% ethanol. This gave (–)-epigallocatechin and 0.223 g of an amorphous substance — a mixture of the two thioethers (**8** and **9**).

Cleavage of the Thioethers from T3. The thioethers (0.223 g) were mixed with 3 ml of ethanol–acetic acid (9:1), Raney nickel catalyst was added, and the whole was kept at 50°C for 2 h. Then it was filtered, and the filtrate was concentrated and chromatographed on Sephadex LH-20 with elution by 80% ethanol. Two compounds were isolated: (–)-epigallocatechin and (–)-epicatechin (**10**), mp 242-243°C, $[\alpha]_D^{23}$ –71° (*c* 0.16; acetone–water (1:1)), λ_{max} 282 mμ (log ε 3.30).

Thiolytic Cleavage of T4. Compound T4 (0.281 g) was cleaved and the reaction products were purified by the method described above. This gave (–)-epigallocatechin and 0.206 g of a mixture of thioethers. Catalytic cleavage of the thioethers (**8** and **11**) and purification of the products led to (–)-epicatechin and (–)-epicatechin 3-O-gallate (**12**), mp 253-255°C, $[\alpha]_D^{23}$ –175° (*c* 0.19; methanol); λ_{max} 284 mμ (log ε 4.05).

Methylation of T3 and T4. The methylation reaction and the detection of the methylated sugars were carried out by the procedure described in [5]. 2,3,4-Tri-O-methyl-*D*-glucopyranose (**15**) was obtained in both cases. TLC of hydrolysates of the permethylates (**13** and **14**) in the chloroform–methanol (12:1) system also showed the presence of one methylated sugar.

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