PROANTHOCYANIDINS OF Polygonum coriarium. I

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Six proanthocyanidins have been isolated from the roots of Polygonium coriarium. The structures of three oligomeric proanthocyanidins have been established: taranin, consisting of [epigallocatechin gallate]-(4β→8)-[epigallocatechin gallate]-(4β→8)-[epigallocatechin-(4β→8)-epigallocatechin$_2$-(4β→8)-epigallocatechin; taranoside A – [epigallocatechin gallate]-7-O-[-(1→6)-β-D-Glcp]$_3$-(4β→8)-[epigallocatechin-(4β→8)-epigallocatechin]$_2$-(4β→8)-gallocatechin; and taranoside B – [epigallocatechin gallate]-7-O-[-(1→6)-β-D-Glcp]$_4$-(4β→8)-[epigallocatechin-(4β→8)-epigallocatechin]$_2$-(4β→8)-epigallocatechin-(4β→8)-[epigallocatechin gallate).

Many researchers have occupied themselves with the investigation of the chemical composition of the tanning knotweed Polygonum coriarium Grig. [taran dubil'nyi] which is widespread in our country [1].

In order to find new physiologically active substances we have investigated little-studied components: the polymeric proanthocyanidins of knotweed roots gathered in the flowering phase in the gorge of the R. Pskem (Tashkent province).

From an alcoholic extract of the roots, by fractionation with respect to polarity, three fractions of proanthocyanidins were obtained: relatively low-molecular mass, oligomeric, and high-molecular mass.

By column chromatography on the sorbents cellulose and Sephadex LH-20, from the combined oligomeric components we isolated six individual proanthocyanidins with different degrees of polymerization. As a result of the study of the physicochemical properties of the substances isolated we have established the structures of three compounds.

Compound (I), the molecular mass of which is ~2400, we have called taranin. The absorption maxima in the UV spectrum (210, 220, 245, 278, 310 nm) and in the UV spectra (3400, 1695, 1615, 1545, 1450, 1320, 1250, 1040 cm^{-1}) showed the presence in the taranin molecule of aromatic rings present in condensed tannide structures and of an α-carboxylic ester group of an aromatic acid.

The ^{13}C NMR spectra of taranin obtained under conditions of complete suppression of spin–spin coupling of protons (Table 1) showed the characteristic signals only of (–)-epigallocatechin and of a gallic acid residue.

The resonance signals from the C-5, C-7, and C-9 carbon atoms of the phloroglucinol nucleus – ring A – appeared in the form of a broadened signal at 159.0 ppm. An intense signal at 146.3 ppm related to C-3' and C-4' of ring B of gallocatechin. The C-4' atom was screened and, as the result of a diamagnetic shift, resonated in the 133.0 ppm region. The signals from carbon atoms C-2' and C-6' of ring B and the substituted C-8 of the phloroglucinol nucleus coincided and gave a relatively strong signal at 107.0 ppm.

The resonance of signals at 101.5 and 103.2 ppm, assigned to the "upper" C-10 carbon atoms adjacent to the substituted C-4 carbon atoms showed the type of interflavan bond as C-4→C-8 [2].

Analysis of the chemical shifts of the C-2, C-3, and C-4 carbon atoms showed that some of the (–)-epigallocatechin blocks of the chain were galloylated [3-5]. In the spectrum, in addition to the resonance signals of the carbon atoms of the gallic acid residue, a para-

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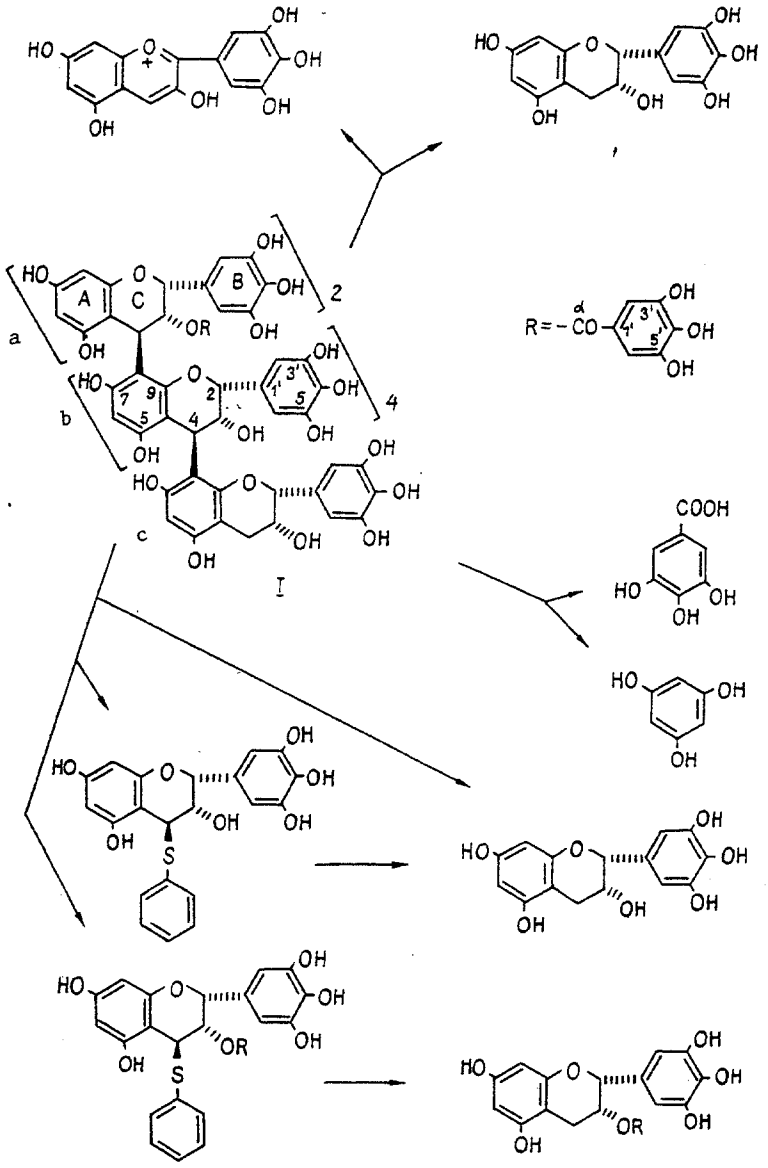
magnetic shift of the C-3 atom and a diamagnetic shift of the C-4 carbon atom of the "upper" epigallocatechins was observed. The appearance of a signal from C-3 at 67.0 ppm and the absence of a C-4 signal at ~26-27 ppm permitted the conclusion that the "lower" epigallocatechin was not esterified. Thus, on the basis of the UV and IR spectra and of an accurate assignment of the signals in the ^{13}C NMR spectrum we concluded that taranin has with most probability structure and configuration (I). Alkaline and mild stepwise thiolytic cleavage of taranin confirmed the facts given above.



Scheme 1

Compound (II) had a molecular mass of ~2500. On the basis of the results of spectral (UV, IR, ^{13}C NMR) and chemical methods of investigation (methylation, acid, thiolytic, and enzymatic cleavages), the substance was assigned to the glycosylated proanthocyanidins, and it has been called taranoside A.

The ^{13}C NMR spectrum of taranoside A (Table 2) showed signals from (−)-epigallocatechin, (+)-gallocatechin, gallic acid residues, and a (1→6)-β-glucan [6, 7]. As analysis of the spectrum showed, one of the "upper" epigallocatechin blocks was galloylated, which was indicated by signals with characteristic chemical shifts − 74.9 ppm (C-3) and 34.7 ppm (C-4) [8]. The presence of a signal at 80.3 ppm, which is characteristic for gallocatechins having the 2,3-trans-configuration of the substituents, showed that the taranoside A molecule contained a gallocatechin block probably occuppying the "lower" position [2].

TABLE 1. Parameters of the ^{13}C PMR Spectra of Taranin, ppm, 0 – TMS

Taranin fragments	Carbon atoms								
	2	3	4	5	6	7	8	9	10
a	75,1	75,1	34,8	159,0	95,0	159,0	95,0	159,2	101,3
b	76,7	72,6	37,0	159,0	95,0	159,0	107,0	159,0	103,2
c	79,1	67,0	*	159,0	95,0	159,0	107,0	159,0	100,5

Taranin fragments	Carbon atoms						
	1′	2′	3′	4′	5′	6′	α
a	131,5	107.0	146,3	133,0	146,3	107,0	
b	131,5	107,0	146,3	133,0	146,3	107,0	
c	131,5	107,0	146,3	133,0	146,3	107,0	
Galloyl	122,5	110,3	146,3	139,1	146,3	110,3	168,2 171,4

*Signal not observed because of overlapping with a signal of the solvent.

TABLE 2. Parameters of the ^{13}C NMR Spectrum of Taraside A, ppm, 0 – TMS

Fragments of taranoside A	Carbon atoms								
	2	3	4	5	6	7	8	9	10
a	74,2	74,9	34,7	158,3	95,1	158,3	95,1	158,3	102,6*
b	76,0*	72,9	36,7	158,3	95,1	158,3	108,6	158,3	104,2*
c	80,3	66,9	†	158,3	95,1	158,3	108,6	158,3	101,2*

Fragments of taranoside A	Carbon atoms						
	1′	2′	3′	4′	5′	6′	α
a	132,4	108,6	146,2	134,2	146,2	108,2	
b	132,4	108,6	146,2	134,2	146,2	108,2	
c	132,4	108,6	146,2	134,2	146,2	108,2	
Galloyl	122,2	111,5	146,2	139,9	146,2	111,5	167,0
Glucan: a	101,2*	74,9	76,0*	71,7	76,0	66,9	
b	102,6*	74,9	76,0*	71,7	76,0	64,6	
c	104,2*	74,9	76,0*	71,7	76,0	62,6	

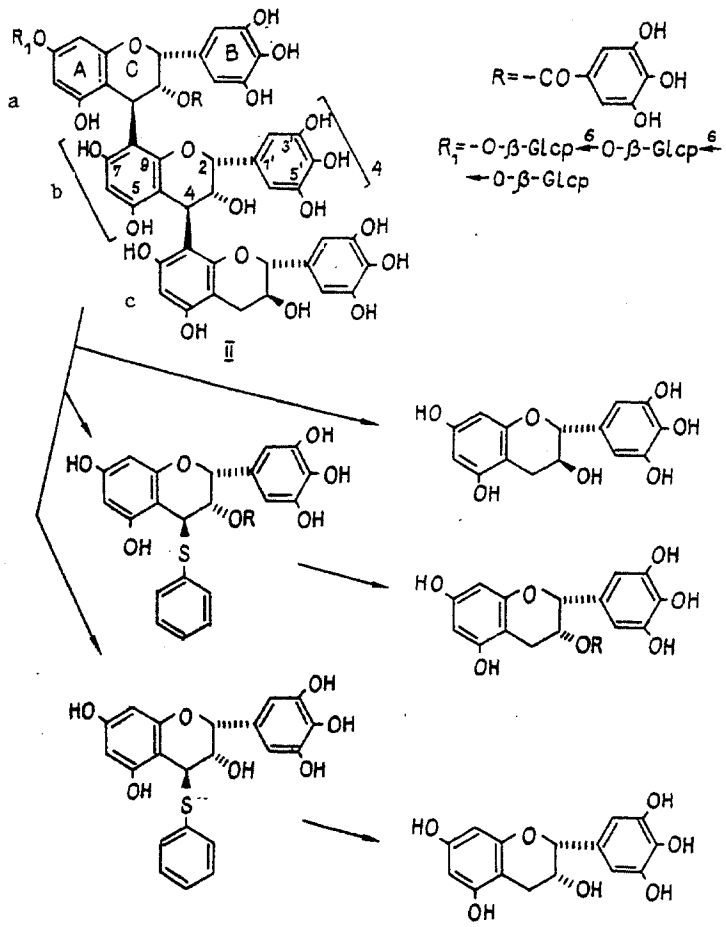
*The signals may be inverted.
†Signal not observed because of overlapping with a signal of the solvent.

The resonance of the C-3 carbon atom of the "lower" block at 66.9 ppm and the simultaneous absence of a signal from C-4 at ~26 ppm gave grounds for stating that the gallocatechin was not esterified. The interflavan bond in taranoside A is realized in the C-4→C-8 manner, as was shown by the C-10 chemical shift (102.6; 104.2 ppm).

In the ^{13}C NMR spectra of taranoside A there were the signals from anomeric carbon atoms at 101.2, 102.6, and 104.2 ppm and from glycosylated carbon atoms at 64.6 and 66.9 ppm, and also from a nonglycosylated carbon atom at 62.6 ppm. The chemical shifts of the C-1, C-3, and C-5 atoms of the glucose residue showed that the anomeric center had the β-configuration [9].

The study of the spectral characteristics and of the products of the enzymatic cleavage of taranoside A and the acid hydrolysis of its permethylate showed that the sugar residue consisted of three D-glucose units linked with the aglycon by a β-glycosidic bond and with one another by (1→6)-β bonds.

In view of the steric hindrance in the taranoside A molecule, the most probable positions of attachment of the sugar residue may be the C-5 position of the "lower" or the C-7



Scheme 2

position of the "upper" gallocatechin blocks. The results of the mild acid and thiolytic cleavage of compound (II) showed the absence of a sugar residue as a component of the "lower" blocks and, consequently, it may be assumed that the "upper" epigallocatechin block was glycosylated.

Compound (III), which we have called taranoside B had a molecular mass of ~3100. On the basis of spectral and chemical methods of investigation it was also assigned to the glycosylated proanthocyanidins.

TABLE 3. Parameters of the ^{13}C NMR Spectrum of Taranoside B, ppm, 0 – TMS

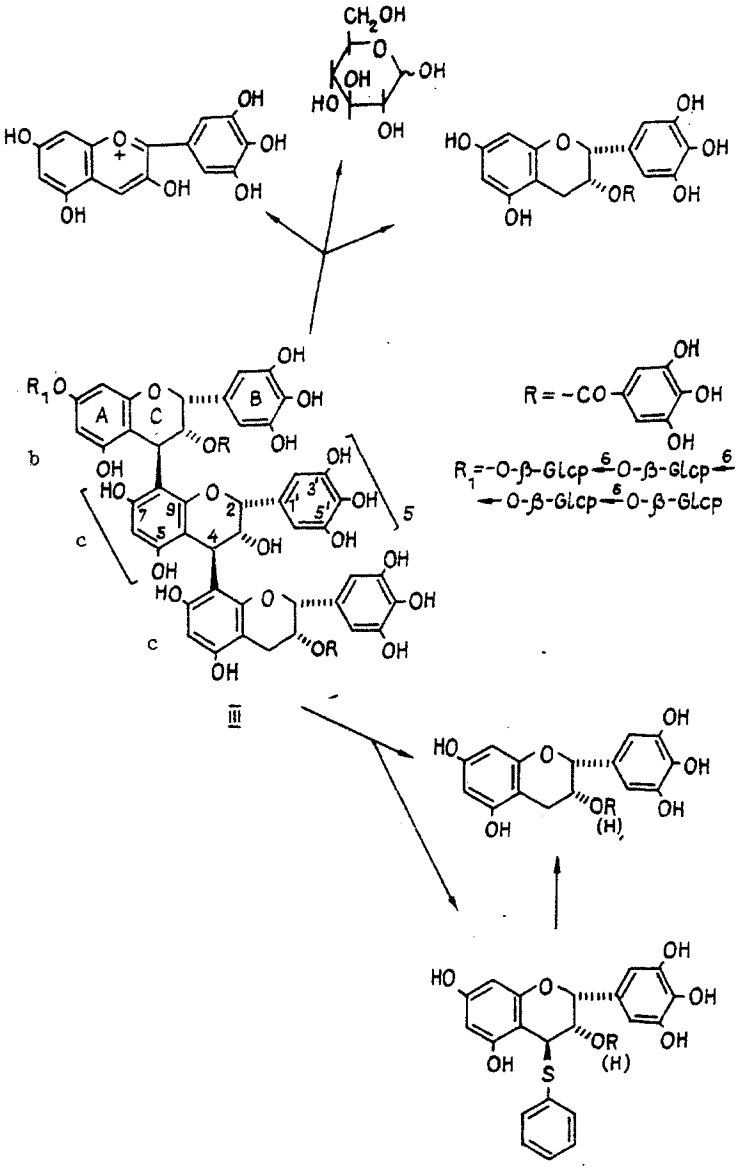
Fragments of taranoside B	Carbon atoms								
	2	3	4	5	6	7	8	9	10
a	75,3	74,6	35,9	157,7	97,5	157,7	97,5	157,7	102,0
b	77,3	71,3	37,7	157,7	97,5	157,7	107,4	157,7	102,0
c	77,3	69,9	26,5	157,7	97,5	157,7	107,4	157,7	101,0

Fragments of taranoside B	Carbon atoms						
	1′	2′	3′	4′	5′	6′	α
a	130,2	107,4	146,1	133,1	146,1	107,4	
b	130,2	107,4	146,1	133,1	146,1	107,4	
c	130,2	107,4	146,1	133,1	146,1	107,4	
Galloyl	121,4	110,9	146,1	139,2	146 1	110,9	170,5
Glucan: a	102,0	74,6	77,3	71,3	76,5	67,0	
b, c	102,0	74,6	77,3	71,3	76,5	64,3	
d	102,0	74,6	77,3	71,3	76,5	62,9	

The ^{13} NMR spectrum of taranoside B was largely analogous to that of taranoside A. The spectrum showed signals relating to (−)-epigallocatechin, to gallic acid residues, and to a (1→6)-β-glucan. The values of the C-2, C-3, and C-4 chemical shifts showed that the "lower" (77.3, 69.9, and 26.5 ppm) and one of the "upper" (75.3, 74.6, and 35.9 ppm) epicatechin blocks was galleoylated. The chemical shifts of the C-10 carbon atom of the "upper" epigallocatechin blocks was characteristic for proanthocyanidins with a C-4→C-8 interflavan bond.

Thus, the spectral results (IR and ^{13}C NMR), the enzymatic cleavage, and the acid hydrolysis of the permethylate of taranoside B showed the presence in the molecule of a glucan consisting of four D-glucose residues linked to the aglycon by a β-glycosidic and with one another by (1→6)-β bonds.

The results of mild and stepwise thiolytic cleavage of (III) showed the absence of a glucan as a component of the "lower" epigallocatechin block and, consequently, if the stereochemical hindrance in the proanthocyanidin molecule is taken into account, as in the case of taranoside A, it is presumably the "upper" epigallocatechin block that is glycosylated.



Scheme 3

EXPERIMENTAL

General Remarks. The UV spectra of the proanthocyanidins and their derivatives were taken in alcoholic solution on a Hitachi EPS-3T instrument. IR spectra were taken on a Carl Zeiss, Jena, UR-20 spectrometer using tablets molded with potassium bromide. ^{13}C NMR spectra were taken on a Tesla BS 567A/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 varied within the range of 15-20%. Molecular masses were determined on a MOM 3170 ultracentrifuge and by gel filtration on a calibrated column of Sephadex LH-20. In the determination of the optical activities of the substances we used a Jasco J-20 instrument. For the identification and determination of the homogeneity of the substances we used PC and TLC on Silufol UV-254 plates. Solvent systems: 1) chloroform–n-butanol–acetone–formic acid–water (3.5:13:10:10:8); 2) BAW (4:1:5); and 3) chloroform–n-butanol–acetone–formic acid–water (3.5:12:20:10:8).

Isolation of the Proanthocyanidins. The roots of Polygonum coriarium (5 kg) were extracted with 96% ethanol six times. The extracts were combined and evaporated in vacuum at 40°C to 2.5 liters. The concentrated extract was treated successively with diethyl ether, ethyl acetate, and n-butanol. This gave 12.5, 15.0, and 250 g of extracts. From the aqueous residue was obtained 1400 g of high-molecular-mass proanthocyanidins. The ethyl acetate extract contained 11 proanthocyanidins and the butanol extract contained 14 components.

Separation of the Proanthocyanidins. The butanolic extract (50 g) was mixed with 50 g of cellulose and was transferred to a column of cellulose (450 g). Elution was conducted with chloroform–ethyl acetate (1:10-1:20), ethyl acetate, and acetone, 100-ml fractions being collected. Monitoring was performed by TLC in systems 1 and 3. The eluate fractions 71-145, containing a mixture of three substances, were combined, evaporated, and rechromatographed (8.2 g) on cellulose. Elution was performed with ethyl acetate and with ethyl acetate–acetone (10:1-1.1).

Taranin (I). The residue from fractions 26-30 (1.79 g) was transferred to a column of Sephadex LH-20 (3 × 130 cm) and elution was conducted with 80% ethanol. This led to the isolation of 1.62 g of a light brown amorphous powder with MM ~ 2400, decomposing at 290-300°C, $[\alpha]_D^{22}$ +70° (c 0.32; ethanol), R_f 0.90 (system 1). IR spectrum, cm^{-1}: ν_{max} 3400, 1695, 1615, 1545, 1450, 1320, 1250, 1120, 1040, 830, 810, 775, 740.

Taranoside A (II). The residue (2.51 g) from fractions 34-39 was chromatographed on Sephadex LH-20, with elution by 60% ethanol. This gave 2.42 g of an amorphous substance with MM ~ 2500, decomposing at 290-300°C, $[\alpha]_D^{22}$ +76° (c 0.27; ethanol), R_f 0.74 (system 1). IR spectrum, cm^{-1}: ν_{max} 3400, 1700, 1620, 1545, 1460, 1340, 1250, 1110, 1045, 890, 830, 810, 780, 750.

Taranoside B (III). The residue from fractions 45-53 (1.98 g) was chromatographed on Sephadex H-20 with elution by 60% ethanol. This yielded 1.81 g of an amorphous substance with MM ~ 3100, $[\alpha]_D^{22}$ +60° (c 0.33; ethanol), R_f 0.61 (system 1). IR spectrum, cm^{-1}: ν_{max} 3400, 1690, 1625, 1550, 1460, 1340, 1250, 1110, 1040, 860, 830, 805, 770, 745.

Alkaline Cleavage of (I), (II), and (III). With the passage of a slow current of nitrogen, a mixture of 50 mg of substance with 5 ml of 50% KOH was immersed in a bath having a temperature of 155-160°C, and then the temperature was raised in 5 min to 230°C. The reaction mixture was cooled, acidified, diluted with water, and extracted with ethyl acetate. The extract was dried, the solvent was distilled off, and the residue was chromatographed on polyamide. This gave two compounds: $C_6H_6O_3$, M^+ 126, mp 218-219°C; and $C_7H_6O_5$, M^+ 170, mp 220°C, decomp., which were identified as phloroglucinol and gallic acid, respectively.

Acid Cleavage of (I). A solution of 150 mg of the substance in 4 ml of ethanol was treated with 3 ml of 2 N HCl and was heated on the water bath under reflux in a current of nitrogen for 2 h, the course of the reaction being monitored by TLC every 20 min. The reaction mixture was diluted with water and extracted with ethyl acetate (3 × 2 ml). The extract was washed and dried, and the solvent was distilled off. The residue was chromatographed on Sephadex LH-20, with elution by 60% ethanol. This gave 12 mg of (−)-epigallocatechin, mp 215-216°C, $[\alpha]_D^{20}$ −58° (c 0.13; methanol), λ_{max} 272 nm (log ε 3.10), R_f 0.42 (system 2).

In the hydrolysate, by the PC method, we detected delphinidin, R_f 0.36 (2 N HCl), λ_{max} 554 nm (0.1% HCl in ethanol).

Acid Cleavage of (II). Cleavage was achieved by the method described above, and the following substances were detected: (+)-gallocatechin, mp 182-183°C, $[\alpha]_D^2$ +18° (c 0.1; ethanol–water), λ_{max} 272 nm (log ε 3.09), R_f 0.55 (system 2); delphinindin; and D-glucose [R_f 0.51; n-butanol–pyridine–water (6:4:3) system].

Thiolytic Cleavage of (I). A mixture of 460 mg of taranin and 4 ml of phenyl mercaptan was treated with 2 ml of acetic acid in 10 ml of ethanol, and was then left at room temperature for 48 h. The course of the reaction for the first 10 h was monitored by TLC every hour. The reaction mixture was concentrated, and the oily residue obtained was chromatographed on Sephadex LH-20 with elution by ethanol. This gave 34 mg of (−)-epigallocatechin and 382 mg of an amorphous substance (a mixture of two thioethers).

Cleavage of the Thioethers of (I). The thioethers (382 mg) were mixed with 3 ml of ethanol–acetic acid (9:1), Raney nickel catalyst was added, and the mixture was kept at 50°C for 1 h. It was then filtered, and the filtrate was concentrated and chromatographed on Sephadex LH-20 with elution by 80% ethanol. Two compounds were obtained: 51 mg of (−)-epigallocatechin, and 23 mg of (−)-epigallocatechin gallate, mp 210-212°C, $[\alpha]_D^{21}$ −136° (c 0.45; methanol–water).

Thiolytic Cleavage of (II). Taranoside A (420 mg) was cleaved and the reaction products were purified by the method described above, giving 26 mg of (+)-gallocatechin and 307 mg of a mixture of thioethers. The catalytic cleavage of the thioethers and purification led to 48 mg of (−)-epigallocatechin and 6 mg of (−)-epigallocatechin gallate.

Thiolytic Cleavage of (III). The reaction was performed by the method described above. The compounds obtained were identified as (−)-epigallocatechin and (−)-epigallocatechin gallate.

Preparation of the Permethylate of (II). Taranoside A (500 mg) was methylated by Hakomori's method [10]. The product obtained (660 mg) was transferred to a column, and elution was performed with the benzene–acetone (5:1) system. This led to the isolation of 137 mg of the amorphous permethylate.

The composition of the methylated sugars in the permethylate of taranoside A was determined by a published method [11] on two phases: 20% of poly(butane-1,4-diyl succinate) on Celite (phase 1), and 10% of poly(phenyl ether) 5F4É on a N-AW-HMDS column (phase 2). T_{rel}: 1.0 and 1.42 (phase 1), 1.00 and 1.33 (phase 2) – 2,3,4,6-tetra-O-methyl-D-glucopyranose; and T_{rel}: 3.10 and 3.73 (phase 1); 1.68 (phase 2) – 2,3,4-tri-O-methyl-D-glucopyranose.

The TLC of a hydrolysate of the permethylate in the chloroform–methanol (12:1) system showed the presence of the same methylated sugars.

Methylation of (III). The methylation and the identification of the methylated sugars were carried out by the method described above. 2,3,4-Tri-O-methyl-D-glucopyranose and 2,3,4,6-tetra-O-methyl-D-glucopyranose were obtained.

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