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NEW OLIGOMERIC PROANTHOCYANIDINS FROM BARK OF *Platanus orientalis*

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Two oligomeric proanthocyanidinglycosides were isolated from bark of Platanus orientalis. *Their structures and relative configurations were established as 7-O-β-D-Glcp-(-)-epicatechingallate-(4β-8)-(-)-epicatechin-(4β-8)-(-)-epicatechin-(4β-8)-5-O-β-D-Glcp-epicatechingallate (**Pl-1**) and 7-O-β-D-Glcp→6-O-β-D-Glcp-(-)-epigallocatechingallate-(4β-8)-(+)-catechingallate-(4β-8)-(+)-catechingallate-(4β-8)-(-)-epigallocatechingallate-(4α-8)-(-)-epicatechin-(4β-8)-[5-O-β-D-Glcp-→6-O-β-D-Glcp→6-galloyl(-)-epigallocatechingallate (**Pl-7**).*

Key words: *Platanus orientalis,* Platanaceae, oligomeric proanthocyanidins, isolation, structure.

The genus *Platanus* (Platanaceae) is represented by four species in Central Asia [1]. The most widely distributed species is *P. orientalis*, eastern plane tree, the chemistry of which is little studied.

The chemical composition of the leaves has been studied to a comparatively greater extent than that of the trunk bark. Phenolcarboxylic acids and flavonoids (hyperin, tiliroside) were isolated from the leaves. The hydrolysates contained quercetin, myricetin, cyanidin, and delphinidin [2, 3]. Fallen leaves of plane tree are rich in α-tocopherol, its oxidized dimeric forms, and esters with higher fatty acids [4, 5], and carbohydrates [6].

The trunk bark contains neutral phytosterols (sito- and stigma-), α-tocopherol, and polyprenols [7] in addition to triterpenoids such as betulinic acid, betulinic aldehyde, platanoic and 3-dehydroplatanoic acids, and sitosterol [3, 8].

Trunk bark is used in folk medicine to treat dysentery, toothache, diarrhea, etc. [9, 10] and as an anticancer agent [11].

The goal of the present work was to investigate the chemical composition including proanthocyanidins of plane-tree trunk bark.

Proanthocyanidins were isolated by fractionating the aqueous-alcohol extract of *P. orientalis* bark according to polarity using organic solvents. This produced low-molecular-weight, oligomeric, and polymeric fractions of proanthocyanidins.

Column chromatography over finely crystalline cellulose and gel-filtration over Sephadex LH-20 isolated from the butanol fraction of the aqueous-alcohol extract two pure oligomeric glycosylated proanthocyanidins **Pl-1** and **Pl-7**, which gave a red color with vanillin-H_2SO_4.

Compound **Pl-1** had empirical formula $C_{86}H_{78}O_{44}$ and molecular weight MW = 1814.

Alkaline decomposition of **Pl-1** under a N_2 atmosphere formed three compounds: fluoroglucinol (**1**) and protocatechoic (**2**) and gallic acids (**3**). Hydrolysis cleaved not only interflavane bonds but also flavan-3-ol units at the pyran heterocycle. Fluoroglucinol formed from ring A; phenolic acid, from ring B. Atoms C_3-C_4 of ring C gave acetic acid [12, 13].

Acid hydrolysis of **Pl-1** cleaved interflavane C–C bonds. This produced (-)-epicatechin-3-O-gallate (**4**), cyanidin (**5**), and glucose (**6**). Thiolytic cleavage of **Pl-1** in the presence of thiophenol and acetic acid formed three compounds. The "lower" part of the molecule gave (-)-epicatechin-3-O-gallate (**4**); the "upper" blocks, a mixture of thioethers **7** and **8**, which then decomposed catalytically in the presence of Raney Ni. The resulting compounds were identified by physicochemical and spectral properties as (-)-epicatechin (**9**) and (-)-epicatechin-3-O-gallate (**4**).

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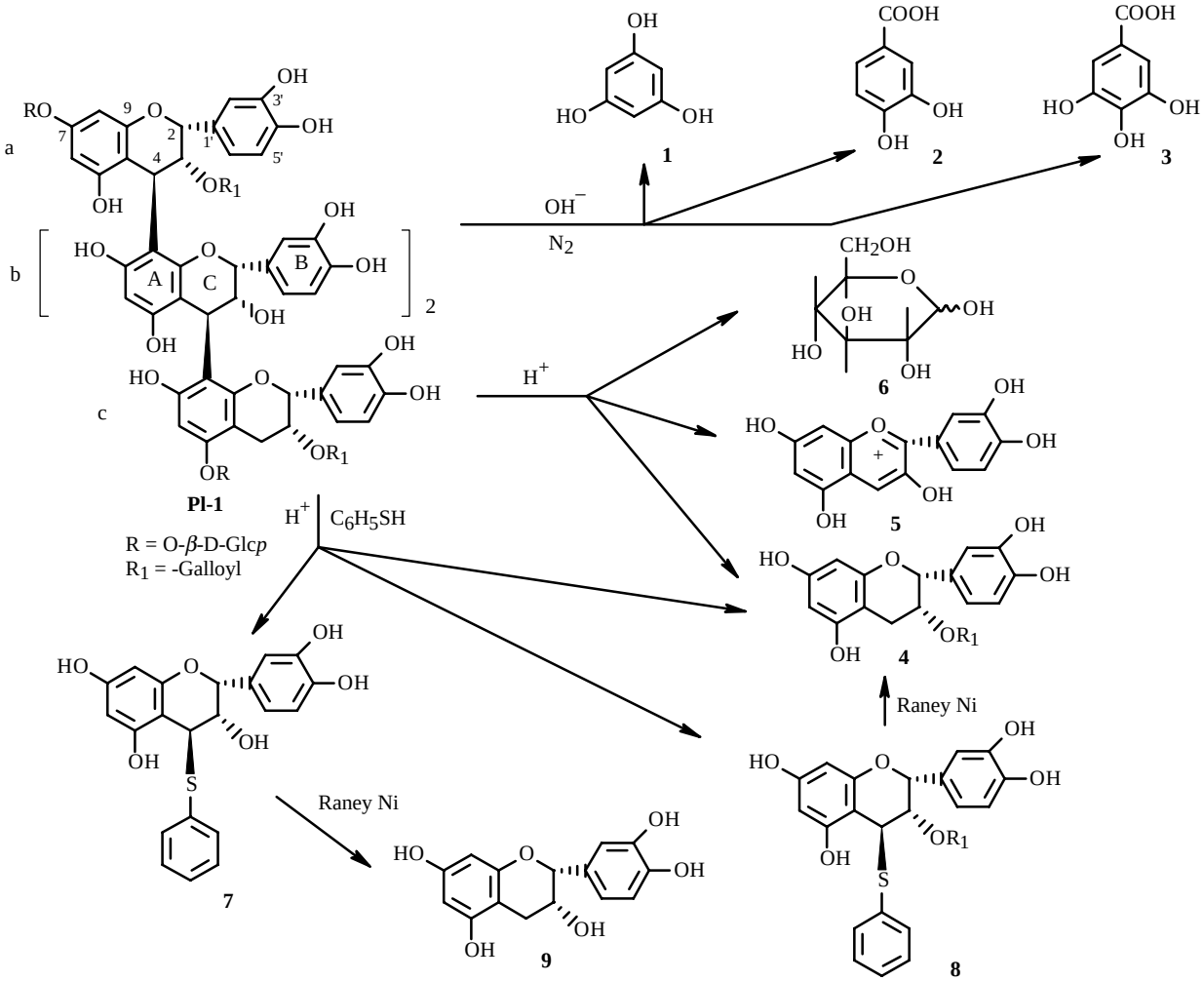
TABLE 1. Chemical Shifts (ppm) of C Atoms in the ^{13}C NMR Spectrum of **Pl-1**

C atom	**Pl-1** fragment				
	a	b	c	Galloyl	Glucose
2	74.53	76.24*; 76.49*	76.49		
3	74.53	71.91; 73.73	69.89		
4	34.37	36.85; 37.82	-		
6	95.36	95.36	95.36		
8	95.36	106.28	106.28		
10	102.21[a]	102.45[a]; 103.43[a]	102.94[a]		
5,7,9	154.90	154.90	154.90		
1′	130.6	130.6	130.6	123.97	103.16[a]; 104.38[a]
2′	114.97	114.97	114.97	109.08	74.53
3′	115.50	115.50	115.50	144.50	76.49*
4′	144.50	144.50	144.50	142.55	70.13
5′	144.50	144.50	144.50	144.50	76.24*
6′	119.04	119.04	119.04	109.08	61.36; 62.59
-COO-				167.28; 170.88	

[a]Signals with the same superscripts may be inverted.

*Signal not observed due to overlap with solvent signal.



Scheme 1

TABLE 2. Chemical Shifts (ppm) of C Atoms in the ^{13}C NMR Spectrum of **Pl-7**

C atom	**Pl-7** fragment						
	a	b	c	d	e	Galloyl	Glucose
2	74.85[a]	81.48; 82.46	74.85[a]	76.24	76.24		
3	74.85[a]	73.42; 73.57	74.85[a]	70.85	70.85		
4	34.10	37.01[e]	34.10	37.01[e]	25.45		
6	97.32	96.57	96.57	96.57	96.57		
8	96.57	107.36[c]	108.03[c]	107.36[c]	108.03[c]		
10	101.90[b]	102.14[b]	103.16[b]	103.16[b]	102.52[b]		
5,7,9	154.46	154.46	154.46	154.46	154.46		
1′	130.98	130.98	130.98	130.98	130.98	120.74[f]	103.16[b]
2′	107.37[c]	116.14	107.37[c]	116.14	107.37[d]	110.01	74.83[a]; 74.45[a]
3′	144.78	144.78	144.78	144.78	147.87	144.78	76.24
4′	133.24	144.78	133.24	144.78	133.24	139.45	70.85
5′	144.78	116.14	144.78	116.14	147.87	144.78	76.75; 78.48
6′	107.37[d]	119.99[f]	107.37[d]	119.99[f]	108.03[d]	110.01	65.70
6′-(Gall)		catechin					63.13
6 (OH)							60.75

Signals with the same superscripts may be inverted.

Enzymatic hydrolysis of **Pl-1** by β-glucosidase produced β-glucopyranose units.

According to the ^{13}C NMR spectrum, **Pl-1** was a glycosylated proanthocyanidin.

The ^{13}C NMR spectrum of **Pl-1** recorded with full H—H decoupling exhibited signals for catechins with the 2,3-*cis*-configuration (lack of signals at 80-83 ppm, typical of 2,3-*trans*-blocks). The chemical shifts of ring-C C atoms (C-2, C-3, C-4) were similar to those of galloylated and ungalloylated epicatechins in proanthocyanidins. Table 1 shows that the signals at 154.90 ppm belong to O-substituted C-5, C-7, and C-9 of ring A.

Ring-A atoms C-6 and C-8 are free of interflavane bonds and appeared at 95.36 ppm. Atoms involved in interflavane bonds appeared at 106.28 ppm [14].

The interflavane bond in **Pl-1** is of the C-4–C-8 type.

Analysis of the chemical shifts of ring-B C atoms showed that **Pl-1** consists only of epicatechin units. The strong signal at 144.50 ppm belongs to substituted C-3′ and C-4′ of ring B. The resonance at 114.97 ppm is typical of C-2′; at 115.50 and 119.04 ppm, of C-5′ and C-6′. The signal at 130.6 ppm belongs to C-1′ [15, 16].

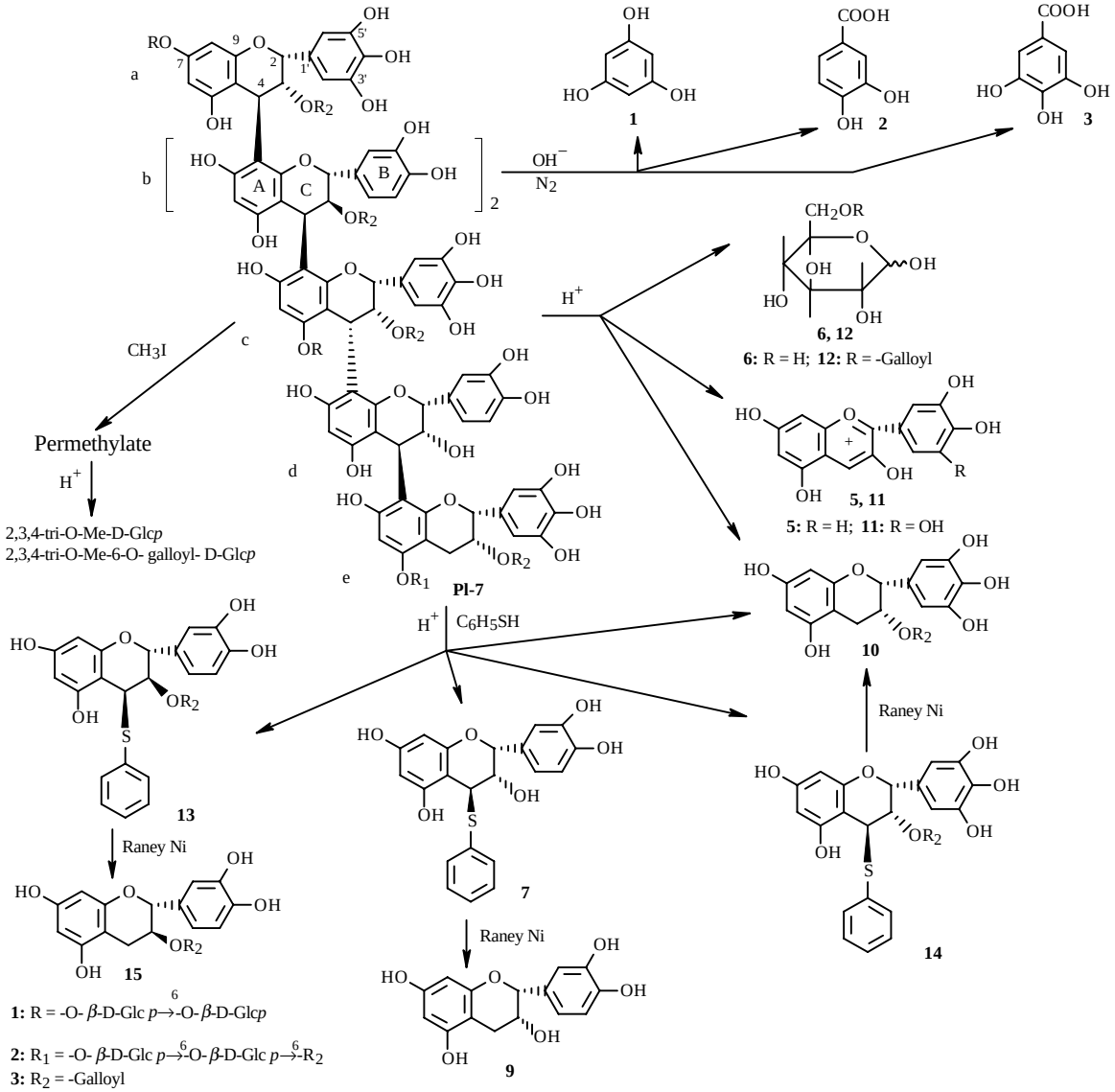
The appearance of signals for C-2 of ring C at 74.53-76.49 ppm indicates that the flavan-3-ols in the proanthocyanidin have the 2,3-*cis*-configuration at the asymmetric centers [16, 17].

Furthermore, the spectrum of **Pl-1** exhibited signals for β-glucose and gallic acid. The principal signals for glucose overlapped those of C atoms in heterocyclic ring C of the proanthocyanidin. This indicated unambiguously that this part was galloylated. The signal of the sixth C atom of glucose (61.36, 62.39 ppm) is typical of the sugar unit in this instance.

Based on the splittings in the ^{13}C NMR and a comparison of them with the literature, we established the structure and relative configuration of the isolated oligomeric proanthocyanidinglycoside **Pl-1** as 7-O-β-D-Glc*p*-(-)-epicatechingallate-(4β-8)-(-)-epicatechin-(4β-8)-(-)-epicatechin-(4β-8)-5-O-β-D-Glc*p*-epicatechingallate.

Compound **Pl-7** has empirical formula $C_{156}H_{138}O_{87}$, M^+ = 3402, and is an oligomeric proanthocyanidinglycoside.

Its structure was established using chemical fragmentation methods for proanthocyanidins (Scheme 2). Alkaline hydrolysis of **Pl-7** formed fluoroglucinol (**1**) and protocatechoic (**2**) and gallic acids (**3**). Acid hydrolysis of **Pl-7** gave (-)-epigallocatechin-3-O-gallate (**10**), delphinidin (**11**), cyanidin (**5**), glucose (**6**), and galloylglucose (**12**). Decomposition of **Pl-7** by thiophenol in acidic medium produced (-)-epigallocatechin-3-O-gallate (**10**) and thioethers **13**, **7**, and **14**. Cleavage of the mixture of thioethers by Raney Ni produced (+)-catechin-3-O-gallate (**15**), (-)-epicatechin (**9**), and (-)-epigallocatechin-3-O-gallate (**10**). Enzymatic hydrolysis of **Pl-7** by β-glucosidase gave glucose and galloylglucose. Methylation of **Pl-7** with subsequent acid hydrolysis produced 2,3,4-tri-O-Me-D-Glc*p* and 2,3,4-tri-O-Me-6-O-galloyl-D-Glc*p*.



Scheme 2

The ^{13}C NMR spectrum of **Pl-7** contains signals for epicatechin and epigallocatechin, gallic acid, and glucose (Table 2).

The distribution of the C chemical shifts for ring C indicates that the proanthocyanidin consists of gallocatechin and catechin blocks with the 2,3-*cis*-configuration, a large part of which is galloylated (34.10, 25.45 ppm, C-4).

The interflavane bonds in **Pl-7** are of the C-4–C-8 type.

In most instances, the sugar signals overlap those of the C atoms in ring C. Only the signal for glucose C-6 lies out of this region. One of these appears at 65.7 ppm, which indicates that this position is glycosylated. The characteristic signal of C-6 at 63.13 ppm indicates that the terminal carbohydrate unit of one of the glucan chains is galloylated in this position. The ^{13}C NMR spectrum and enzymatic hydrolysis showed that **Pl-7** contains two glucan chains. The most probable sites of attachment to the proanthocyanidin, considering stereochemical hindrance, are C-7 of the upper blocks and C-5 of the lower blocks in **Pl-7**.

Based on spectral and chemical data, we propose for **Pl-7** the structure and relative configuration 7-O-β-D-Glc*p*→6-O-β-D-Glc*p*-(-)-epigallocatechingallate-(4β-8)-(+)-catechingallate-(4β-8)-(+)-catechingallate-(4β-8)-(-)-epigallocatchingallate-(4α-8)-(-)-epicatechin-(4β-8)-5-O-β-D-Glc*p*→6-O-β-D-Glc*p*→6-galloyl-(-)-epigallocatechingallate.

EXPERIMENTAL

UV spectra of proanthocyanidins and their derivatives were recorded in alcohol solution on a Lambda-16 (Perkin-Elmer) instrument; IR spectra, on a System 2000 FT-IR (Perkin-Elmer) instrument as KBr disks.

^{13}C NMR spectra of **Pl-1** and **Pl-7** were recorded on an AM 400/400 MHz (Bruker) instrument in a mixture of $(CD_3)_2CO$ and D_2O.

Molecular weights were determined on a MOM 3170 ultracentrifuge and by gel chromatography over a calibrated column of Sephadex LH-20. TLC on Silufol UV-254 plates was used to determine the purity of the compounds.

The following solvent systems were used: $C_4H_9OH:CH_3CO_2H:H_2O$ (4:1:5, 1; 40:12:28, 2), $CHCl_3:CH_3OH:H_2O:CH_3CO_2H$ (9:3:0.5:0.5, 3), $CHCl_3$:*n*-$C_4H_9OH:(CH_3)_2CO:HCO_2H:H_2O$ (3.5:13:10:10:8, 4).

Elemental analyses of all compounds agreed with those calculated.

Extraction and Isolation of Proanthocyanidins. Ground and air-dried bark of *P. orientalis* (5.0 kg) was extracted with ethanol (80%, 6×20 L). The resulting ethanol extracts were combined. The alcohol was vacuum distilled at 50-55°C. The remaining thick extract (350.2 g, 7% calculated for air-dried raw material) was diluted with distilled water (1:1 by vol) and fractionated successively by polarity using organic solvents: hexane (500×4) to remove low-molecular-weight slightly polar compounds, ethylacetate (500×4) to extract monomeric and dimeric proanthocyanidins, and *n*-butanol (500×4) to remove relatively moderately polymerized proanthocyanidins and their glycosides.

Then, the hexane, ethylacetate, and butanol extracts were evaporated to afford slightly polar (65.2 g, 1.3%), relatively moderately polar (17.3 g, 0.34%), and polar total compounds (32.9 g, 0.7%), respectively. After isolating these compounds, the remaining aqueous solution was evaporated in a porcelain dish on a water bath with constant stirring, drying, and grinding to afford total high-molecular-weight proanthocyanidins (234.7 g, 4.7%) of light brown color.

Separation of Proanthocyanidins. The butanol extract (32.9 g) was mixed with cellulose (32.9 g) and placed on a column of microcrystalline cellulose (140×6 cm, 1800 g) and eluted with $CHCl_3$, $CHCl_3:C_2H_5O_2CCH_3$, $C_2H_5O_2CCH_3$, $C_2H_5O_2CCH_3:(CH_3)_2CO$, $(CH_3)_2CO$, and $(CH_3)_2CO:H_2O$. Fractions of 100 mL were collected. The elution was monitored using TLC. Homogeneous fractions (TLC monitoring) were combined and rechromatographed over a column of Sephadex LH-20 (140×3 cm, 158 g).

Proanthocyanidin Pl-1, 0.530 g, $C_{86}H_{78}O_{44}$, M^+ 1814, $[\alpha]_D^{16}$ +36.2° (acetone:water, 1:1).

UV spectrum (nm, λ_{max}): 211, 275; (λ_{min}) 240.

IR spectrum (ν_{max}, cm^{-1}): 3394, 1716, 1616, 1521, 1456, 1374, 1286, 1119, 1071.

Table 1 lists the ^{13}C NMR spectral data.

Proanthocyanidin Pl-7, 0.710 g, $C_{156}H_{138}O_{87}$, M^+ 3402, $[\alpha]_D^{16}$ +55.5° (acetone:water, 1:1).

UV spectrum (nm, λ_{max}): 233, 273; (λ_{min}) 250.

IR spectrum (ν_{max}, cm^{-1}): 3414, 3399, 1717, 1616, 1522, 1456, 1201, 1038.

Table 2 lists the ^{13}C NMR spectral data.

Alkaline Cleavage of Pl-1 and Pl-7. Pl-1 (70 mg) or **Pl-7** (80 mg) was constantly stirred under a stream of N_2 and treated with KOH solution (5 mL, 50%). The lower part of the flask was immersed in a bath with a low-melting metal alloy at 150-160°C. The bath temperature was increased over 5 min to 230°C. The reaction mixture was quickly cooled by immersing the flask in icewater, acidified with H_2SO_4 (20%), diluted with water, and extracted with ethylacetate. The ethylacetate extract was dried over anhydrous Na_2SO_4. The solvent was removed. The solid was chromatographed over a polyamide column to afford fluoroglucinol (**1**) and catechoic (**2**) and gallic acids (**3**) [11].

Acid Cleavage of Pl-1. Pl-1 (80 mg) was dissolved in ethanol (4 mL), treated with HCl solution (1.5 mL, 2 N), and refluxed on a water bath for 2 h under a stream of N_2. The reaction mixture was diluted with water and extracted with ethylacetate. The extract was washed with $NaHCO_3$ solution and dried over anhydrous Na_2SO_4. The solvent was removed. The solid was chromatographed over a column of Sephadex LH-20 to afford (-)-epicatechin-3-O-gallate {**4**, 4 mg, $C_{22}H_{18}O_9$, M^+ 426, mp 210-211°C, $[\alpha]_D^{22}$ -135° (*c* 0.38, methanol:water)} and cyanidin (**5**) and to detect D-glucose (**6**).

Acid cleavage of Pl-7 (100 mg) was performed analogously to afford (-)-epigallocatechin-3-O-gallate {**10**, 6 mg, $C_{22}H_{18}O_{11}$, M^+ 458, mp 210-212°C, $[\alpha]_D^{22}$ +184.8° (*c* 0.89, water)}, cyanidin (**5**), delphinidin (**11**), galloylglucose (**12**), and glucose (**6**).

Thiolytic Cleavage of Proanthocyanidins. Cleavage of Pl-1. Pl-1 (210 mg) and thiophenol (5 mL) were mixed, treated with acetic acid (3 mL) in ethanol (10 mL), and left at room temperature for 48 h. The course of the reaction was

monitored using TLC. The reaction mixture thickened. The resulting oily residue was chromatographed over Sephadex LH-20 with elution by ethanol to afford (-)-epicatechin-3-O-gallate (**4**, 8.5 mg) and an amorphous mixture of two thioethers (95 mg).

Thioether Cleavage. Thioethers (95 mg) were mixed with ethanol:acetic acid (9:1, 4 mL), treated with catalyst (Raney Ni), held for 1 h at 50°C, and filtered. The filtrate was condensed and chromatographed over Sephadex LH-20 with elution by ethanol (80%) to afford two compounds: (-)-epicatechin (**9**) and (-)-epicatechin-3-O-gallate (**4**).

Pl-7 Cleavage. The compound (300 mg) was cleaved and the reaction products were purified as described above. The reaction mixture was chromatographed over Sephadex LH-20 with elution by ethanol (60%) to afford (-)-epigallocatechin-3-O-gallate (**10**, 12.6 mg) and a mixture of thioethers (132 mg).

Thioether Cleavage. Thioethers (132 mg) were mixed with ethanol:acetic acid (9:1, 2.5 mL), treated with catalyst (Raney Ni), held for 1 h at 50°C, and filtered. The filtrate was condensed and chromatographed over Sephadex LH-20 with elution by ethanol (80%) to afford (+)-catechin-3-O-gallate (**15**), (-)-epicatechin (**9**), and (-)-epigallocatechin-3-O-gallate (**10**).

Pl-7 Methylation. A solution of **Pl-7** (55 mg) and DMSO (10 mL) was stirred, treated with NaH (0.10 g), held for 1 h at room temperature, treated dropwise with CH_3I (5 mL), left another 4 h, poured into icewater (30 mL), and extracted with $CHCl_3$. The extract was treated with sodium thiosulfate, washed with water, and dried over anhydrous Na_2SO_4. The solvent was removed. The resulting solid was methylated another five times. The final product was purified by column chromatography to afford an amorphous permethylate (44 mg).

Hydrolysis of Pl-7 Permethylate. The resulting permethylate (44 mg) was dissolved in methanol (50%, 5 mL) 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 methylated carbohydrates (21 mg) that were identified as 2,3,4-tri-O-methyl-D-glucopyranose and 2,3,4-tri-O-methyl-6-O-galloyl-D-glucopyranose by comparison with authentic samples.

Enzymatic Hydrolysis of Pl-1 and Pl-7. Glycoside (15 mg) was dissolved in water (10 mL) and treated with β-glucosidase. The reaction mixture was placed in a thermostat and held for 6 h at 30°C. Polyphenols were precipitated by lead acetate solution. Glucose was detected in the filtrate by paper chromatography.

REFERENCES

1. *Plant Resources* [in Russian], Nauka, Leningrad (1985), p. 124.
2. E. C. Bate-Smith, *J. Linn. Soc. London Bot.*, **58**, No. 371, 95 (1962).
3. R. Hegnauer, *Chemotaxonomie der Pflanzen*, Basel, Stuttgart (1969), Vol. 5, p. 506.
4. U. A. Abdullaev, Ya. V. Rashkes, N. K. Khidyrova, and A. M. Rashkes, *Khim. Prir. Soedin.*, 363 (1994).
5. N. K. Khidyrova, Ya. V. Rashkes, A. M. Rashkes, U. A. Abdullaev, M. T. Khodzhaeva, Kh. M. Shakhidoyatov, and M. T. Turakhozhaev, *Khim. Prir. Soedin.*, 372 (1995).
6. M. A. Khodzhaeva, M. T. Turakhozhaev, N. K. Khidyrova, A. M. Rashkes, Ya. V. Rashkes, and Kh. M. Shakhidoyatov, *Khim. Prir. Soedin.*, 619 (1995).
7. S. Z. Nishanbaev, S. E. Kiyamova, N. K. Khidyrova, Z. A. Kuliev, and Kh. M. Shakhidoyatov, *Program and Abstracts of Papers of the Conference of Young Scientists* [in Russian], Tashkent (2003), 51.
8. R. T. Alpin, T. G. Halsall, and T. Norin, *J. Chem. Soc.*, 3269 (1963).
9. H. A. Hoppe, *Drogenkunde*, 1231 (1958).
10. R. N. Chopra, S. L. Nayar, and J. K. Chopra, *Glossary of Indian Medicinal Plants*, (1956), 330.
11. J. K. Hartwell, *Lloydia*, **33**, No. 1, 97 (1970).
12. K. H. Kim, Z. A. Kuliev, A. D. Dvodin, M. R. Yagudaev, and V. M. Malikov, *Khim. Prir. Soedin.*, 771 (1991).
13. M. I. Zaprometov, *Principles of Polyphenolic Compounds* [in Russian], Nauka, Moscow (1974), 63.
14. A. D. Vdovin, Z. A. Kuliev, and N. D. Abdullaev, *Khim. Prir. Soedin.*, 545 (1997).
15. D. Sun, H. Wong, and Y. Foo, *Phytochemistry*, **26**, No. 6, 1825 (1987).
16. Z. Czochanska, L. Y. Foo, R. H. Newman, and L. J. Porter, *J. Chem. Soc., Perkin Trans. 1*, 2278 (1980).
17. F. Hsu, G. Nonaka, and I. Nishioka, *Chem. Pharm. Bull.*, **33**, No. 8, 3293 (1985).