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NEW OLIGOMERIC PROANTHOCYANIDIN GLYCOSIDES PLATANOSIDE-A AND PLATANOSIDE-B FROM *Platanus orientalis* TRUNK BARK

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Two new oligomeric proanthocyanidin glycosides were isolated from trunk bark of Platanus orientalis. *Their structures and relative configurations were found to be 7-*O-β-*D-Glc*p-*(–)-epicatechin-(4*β-*8)-(–)-epicatechin-(4*β-*8)-(–)-epicatechin-3-*O-*gallate (platanoside-A) and 7-*O-β-*D-Glc*p-$\overset{6}{\rightarrow}$*galloyl-(+)-catechin-3-*O-*gallate-(4*α-*8)-(–)-epicatechin-3-*O-*gallate-(4*β-*8)-(–)-epicatechin-3-*O-*gallate-(4*β-*8)-5-*O-β-*D-Glc*p-*(–)-epicatechin-3-*O-*gallate (platanoside-B).*

Keywords: *Platanus orientalis*, Platanaceae, oligomeric proanthocyanidin glycosides, isolation, structure, ^{13}C NMR, chemical transformations.

Plants synthesize numerous compounds of various classes. Some of the most common natural compounds are polyphenols. These include various groups of biologically active compounds that occur in many plants in various combinations and are active principles of greater than 70% of medicinal agents [1]. Among these, plant proanthocyanidins are of great interest because preparations with antioxidant and bactericidal properties for treating atherosclerosis are found in this series [2–5].

We have previously isolated and elucidated the structures of two proanthocyanidins from trunk bark of *Plantanus orientalis* L. (Platanaceae) [6]. In continuation of studies in this area, we isolated two more new proanthocyanidins. For this, the aqueous EtOH extract of *P. orientalis* trunk bark was fractionated according to polarity of the organic solvents to produce fractions of low-molecular-weight, oligomeric, and polymeric proanthocyanidins.

Column chromatography over finely crystalline cellulose and gel filtration over Sephadex LH-20 isolated from the BuOH fraction of the aqueous EtOH extract of *P. orientalis* trunk bark two new pure oligomeric glycosylated proanthocyanidins platanoside-A (**I**) and platanoside-B (**II**) [7, 8].

The elemental composition of **I** was $C_{58}H_{51}O_{27}$; molecular weight MW ~ 1170–1180. UV, IR, and ^{13}C NMR spectroscopy (Table 1) showed that this oligomeric proanthocyanidin consisted of (–)-epicatechin and (–)-epicatechin-3-*O*-gallate.

We determined the monomer composition and established the structure using chemical methods. Thus, the compositions of fragments of catechin blocks were found by carrying out alkaline cleavage, which produced phloroglucinol (**1**) and protocatechoic acid (**2**) (Scheme 1). Hydrolysis caused not only cleavage of interflavane bonds but also decomposition of the pyran heterocycle of flavan-3-ol units.

Phloroglucinol was formed from ring A; phenolic acid, ring B. The C_3–C_4 atoms of ring C gave acetic acid [9].

Acid hydrolysis was carried out in order to determine the monomer composition. The hydrolysate contained (–)-epicatechin-3-*O*-gallate (**6**), cyanidin (**5**), and D-glucose (**4**) [6].

The terminal fragments of the molecule were found by using thiophenol cleavage [6]. Treatment of **I** with thiophenol formed from its lower half (–)-epicatechin-3-*O*-gallate (**6**); from the upper, thioether **7**. Cleavage of thioether **7** in the presence of Raney Ni produced (–)-epicatechin (**8**).

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TABLE 1. Chemical Shifts (ppm) of Resonances in ^{13}C NMR Spectra of Platanoside-A (**I**) and Platanoside-B (**II**)

C atom	δ_C of fragments (**I**)				
	a	b	d	Glucose	Galloyl
2	76.46	76.46	76.46		
3	73.77	73.77	69.79*		
4	36.75	36.75	–		
6	94.53	94.53	94.53		
8	94.53	107.50	107.50		
10	103.2–104.4	103.2–104.4	103.2–104.4		
5, 7, 9	148.0;153.1	148.0;153.1	148.0;153.1		
1′	130.66	130.66	130.66	103.2–104.4	120.0
2′	115.98	115.98	115.98	73.77	111.80
3′	144.59	144.59	144.59	76.46	144.59
4′	144.59	144.59	144.59	69.83*	–
5′	115.98	115.98	115.98	76.46	144.59
6′	119.20	119.20	119.20	61.33	111.80
-COO-					174.50
C atom	δ_C of fragments (**II**)				
	a	b, c	d	Glucose	Galloyl
2	81.40 82.28	74.36	76.46		
3	73.77	74.36	69.79		
4	36.75	34.18	–		
6	94.58	94.53	94.53		
8	92.56	107.50	107.50		
10	102.08	102.08	102.08[a]		
5, 7, 9	153.2–156.7	153.2–156.7	153.2–156.7		
1′	130.91	130.91	130.91	102.08[a]	118.3–121.4
2′	115.5–116.5	115.5–116.5	115.5–116.5	73.77	111.80
3′	143.6–145.7	143.6–145.7	143.6–145.7	76.46	144.59
4′	143.6–145.7	143.6–145.7	143.6–145.7	69.83	139.19
5′	115.5–116.5	115.5–116.5	115.5–116.5	76.46	144.59
6′	118.3–121.4	118.3–121.4	118.3–121.4	61.36 : 63.06	111.80
-COO-					176.10

*Resonances of C-3 of the lower block and C-4 of glucose may be reversed.
[a]Resonances denoted by the same superscripts may be reversed.

Enzymatic analysis by β-glucosidase of **I** indicated that it contained a β-glucopyranose unit.

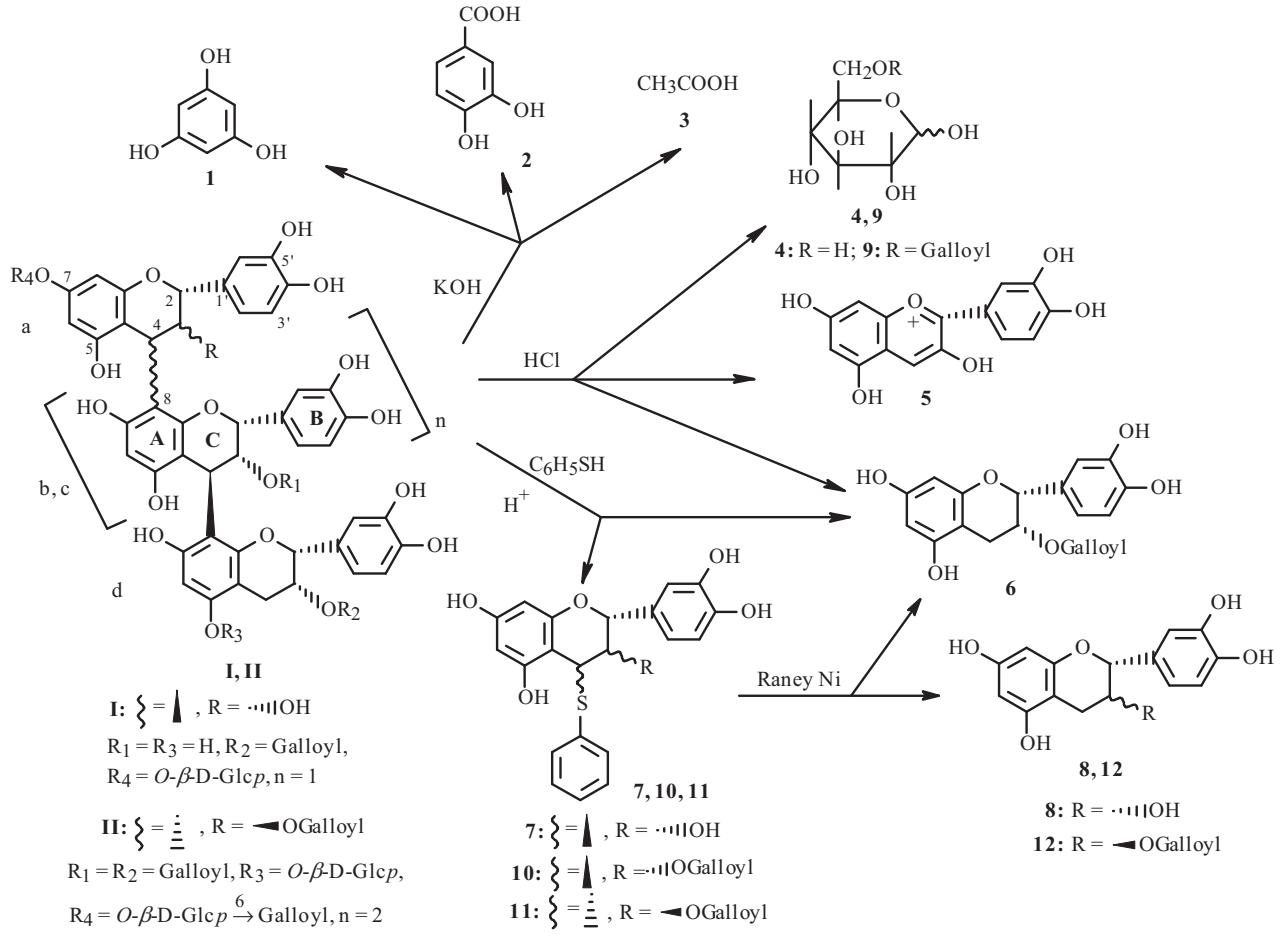
Resonances of (–)-epicatechin, (–)-epicatechin-3-*O*-gallate, glucose, and gallic acid could be fully assigned by interpreting the ^{13}C NMR spectrum (Table 1).

Table 1 shows that resonances at 148.0 and 153.1 ppm belonged to C-5, C-7 of ring A, and C-9 of ring C, which contained an oxygen. Resonances of C-6 and C-8 of ring A that were not involved in interflavane bonds appeared at 94.53; of C-8, which did form an interflavane bond, at 107.50 [10].

An analysis of the chemical shifts of C atoms in ring B found that **I** consisted of only (–)-epicatechin flavane units. The characteristic combination of resonances for C-2′, C-5′, and C-6′ of ring B at 115.98 and 119.20 ppm were indicative of the catechin block [11, 12].

The appearance of resonances for C-2 of ring C at 76.46 ppm indicated that the proanthocyanidin had the 2,3-*cis*-configuration of flavan-3-ols [13, 14].

The chemical shift of C-10 of the proanthocyanidin blocks that was observed at 103.2–104.4 ppm indicated that the proanthocyanidin included a C-4–C-8 interflavane bond [10].



Scheme 1

Interpretation of the carbohydrate resonances of **I** suggested that the chemical shifts of glucose C-1, C-3, and C-5 (103.2–104.4, 76.46, and 76.46) were characteristic of β-D-glucopyranose [15, 16].

Based on the chemical transformations, UV and IR spectra, and assignment of resonances in the ^{13}C NMR spectrum, it was concluded that **I** had the sructure and configuration 7-*O*-β-D-Glc*p*-(–)-epicatechin-(4β-8)-(–)-epicatechin-(4β-8)-(–)-epicatechin-3-*O*-gallate.

We called the second proanthocyanidin platanoside-B (**II**). Its elemental composition was $C_{107}H_{93}O_{55}$; MW, 2250–2260.

According to UV, IR, and ^{13}C NMR spectra (Table 1), **II** was also an oligomeric proanthocyanidin glycoside. Its structure was elucidated using chemical methods for fragmentation of proanthocyanidins (Scheme 1).

Alkaline hydrolysis of **II** formed phloroglucinol (**1**) and protocatechoic (**2**) and acetic (**3**) acids. Acid hydrolysis of **II** gave (–)-epicatechin-3-*O*-gallate (**6**), cyanidin (**5**), glucose (**4**), and galloylglucose (**9**).

Mild thiolytic cleavage in the presence of thiophenol and acetic acid produced from the lower block (–)-epicatechin-3-*O*-gallate (**6**) and thioethers **10** and **11** that were destroyed catalytically in the presence of Raney Ni. The resulting compounds were identified as (–)-epicatechin-3-*O*-gallate (**6**) and (+)-catechin-3-*O*-gallate (**12**).

The ^{13}C NMR spectrum of **II** contained resonances for C atoms of (+)-catechingallate, (–)-epicatechin-3-*O*-gallate, gallic acid, and glucose (Table 1).

Resonances in the range 153.2–156.7 ppm were assigned to C-5, C-7, and C-9 of phloroglucinol ring A. Resonances of unsubstituted C atoms of this ring appeared at 94.58 [10].

Chemical shifts of C atoms in ring B gave a combination of resonances for C-2′, C-5′, and C-6′ at 115.5–116.5, 115.6–116.5, and 118.3–121.4 ppm that were characteristic of only (–)-epicatechin [17].

Resonances of C-2 in fragments of **II** appeared at 82.28, 81.40, and 76.46 ppm. This indicated unambiguously that **II** included both 2,3-*trans* and 2,3-*cis* stereochemistry for the flavan-3-ols [13].

Resonances for heterocyclic C-2 and C-4 in the range 82.28–81.40 and 36.75 in addition to 74.36 and 34.18 ppm indicated that all blocks were substituted by gallate in the C-3 position.

Resonances with CS 102.08 ppm for C-10 were characteristic of proanthocyanidins with a C-4–C-8 interflavane bond [10].

The ^{13}C NMR spectrum of **II** also showed resonances for glucose C atoms. An analysis of the carbohydrate part of the spectrum found that it consisted of two β-D-glucopyranoses, one of which had a gallate unit in the 6-position [10, 17].

Based on the spectral data and chemical transformations, the structure and relative configuration of **II** were proposed as 7-*O*-β-D-Glc*p*$^{6}\rightarrow$galloyl-(+)-catechin-3-*O*-gallate-(4α-8)-(–)-epicatechin-3-*O*-gallate-(4β-8)-(–)-epicatechin-3-*O*-gallate-(4β-8)-5-*O*-β-D-Glc*p*-(–)-epicatechin-3-*O*-gallate.

EXPERIMENTAL

UV spectra of proanthocyanidins and their derivatives in EtOH were recorded on a Perkin–Elmer Lambda-16 instrument; IR spectra, in KBr disks on a Perkin–Elmer System 2000 FT IR insrument; ^{13}C NMR spectra of **I** and **II**, (in deuteroacetone and deuterated water mixtures) on a Bruker AM 400/400 MHz instrument.

Molecular weights were determined using a MOM 3170 ultracentrifuge and a gel-chromatography method over a calibrated column of Sephadex LH-20. Compounds were identified and their purity was determined using PC and TLC on Silufol UV-254 plates. We used solvent systems *n*-BuOH:HOAc:H_2O 4:1:5 (1) and 40:12:28 (2); $CHCl_3$:MeOH:H_2O:HOAc 9:3:0.5:0.5 (3); *n*-BuOH:HCOOH (85%):H_2O 9.5:1:2 (4); HCl (2 N) (5); *n*-BuOH:Py:H_2O 6:4:3 (6). The detectors were vanillin (1%) in alcoholic H_2SO_4 (5%); a mixture of aqueous solutions (1%) of $FeCl_3$ and $K_3[Fe(CN)_6]$ (1:1); and anilinium phthalate.

Extraction and Isolation of Proanthocyanidins. Ground air-dried *P. orientalis* trunk bark (5.0 kg) was extracted with 80% EtOH (25 L and 5 × 20 L). The resulting extracts were combined. The EtOH was vacuum distilled at 50–55°C. The remaining thick extract (350.17 g, 7% of air-dried raw material) was diluted with distilled water (1:1 v/v) and fractionated successively by polarity of the organic solvents hexane (4 × 500 mL) to remove low-molecular-weight slightly polar compounds, EtOAc (4 × 500 mL) to extract monomeric and dimeric proanthocyanidins, and *n*-BuOH (4 × 500 mL) to isolate proanthocyanidins of relatively moderate polymerization and their glycosides.

Then, the hexane, EtOAc, and BuOH extracts were evaporated to afford 65.2 g (1.3%), 17.332 (0.34), and 32.94 (0.7), respectively, total slightly polar, relatively weakly polar, and polar compounds. The remaining aqueous layer was evaporated in a porcelain dish on a water bath with constant stirring, dried, and ground to afford 234.7 g (4.7%) of total high-molecular-weight proanthocyanidins (light-brown solid) [6].

Separation of Proanthocyanidins. The BuOH extract (32.94 g) was mixed with cellulose (32.94 g) and placed onto a column of microcrystalline cellulose (140 × 6 cm, 1800 g). The column was eluted with $CHCl_3$, $CHCl_3$:EtOAc; EtOAc, EtOAc:$(CH_3)_2CO$, $(CH_3)_2CO$, and $(CH_3)_2CO$:H_2O. Fractions of 100 mL were collected. The elution was monitored by TLC. Homogeneous fractions were combined and rechromatographed over a column of Sephadex LH-20 (140 × 3, 158 g). The homogeneity of the fractions was checked by TLC.

Proanthocyanidin I, 0.480 g, $C_{58}H_{51}O_{27}$, MW 1170–1180, $[\alpha]_D^{22}$ ±0° (*c* 0.67, acetone:water, 1:1). UV spectrum (λ_{max}, nm): 279; λ_{min} 260. IR spectrum (ν_{max}, cm^{-1}): 3649, 3264, 1717, 1616, 1522, 1452, 1375, 1286, 1119. Table 1 lists the ^{13}C NMR spectrum.

Proanthocyanidin II, 0.560 g, $C_{107}H_{93}O_{55}$, MW 2250–2260, $[\alpha]_D^{22}$ –59.6° (*c* 0.26, acetone:water, 1:1). UV spectrum (λ_{max}, nm): 277; λ_{min} 259. IR spectrum (ν_{max}, cm^{-1}): 3574, 3543, 1696, 1606, 1506, 1306, 1033. Table 1 lists the ^{13}C NMR spectrum.

Alkaline Cleavage of I. A 20-mL four-necked round-bottomed flask was charged with **I** (65 mg), purged slowly with N_2, and treated with KOH solution (5 mL, 50%). The mixture was constantly stirred. The lower part of the flask was immersed into a bath with a low-melting metallic alloy at 150–160°C that was heated over five minutes to 230°C. The mixture was rapidly cooled by immersing the flask into icewater acidified with H_2SO_4 (20%). The contents of the flask were diluted with water and extracted with EtOAc. The EtOAc extract was dried over anhydrous Na_2SO_4. The solvent was distilled off. The solid was chromatographed over a column of polyamide to afford two compounds with M^+ 126, mp 218–219°C, R_f 0.64 (PC, system 4) (phloroglucinol) and M^+ 154, mp 200°C (dec.), R_f 0.72 (PC, system 4) (protocatechoic acid) [6].

Alkaline cleavage of II (68 mg) was carried out by the method described for **I** to afford two compounds that were identified as phloroglucinol (**1**) and protocatechoic acid (**2**).

Acid Cleavage of 1. Compound **I** (75 mg) was dissolved in EtOH (4 mL), treated with HCl (1.5 mL, 2 N), refluxed under N_2 on a water bath for 2 h, diluted with water, and extracted with EtOAc. The extract was washed with $NaHCO_3$ solution and dried over anhydrous Na_2SO_4. Solvent was distilled off. The solid was chromatographed over a column of Sephadex LH-20 with elution by 60% EtOH to afford a compound (4 mg) of composition $C_{22}H_{18}O_{10}$, mp 253–255°C, $[\alpha]_D^{20}$ –176° (*c* 0.15, MeOH), R_f 0.54 (system 2) [(–)-epicatechin-3-*O*-gallate (**7**)] [6].

Paper chromatography of the hydrolysate detected cyanidin, R_f 0.69 (system 5) and glucose R_f 0.51 (system 6).

Acid cleavage of II (80 mg) was carried out analogously to afford a compound (4.4 mg) of composition $C_{22}H_{18}O_{10}$, mp 253–255°C, $[\alpha]_D^{20}$ –176° (*c* 0.15, MeOH), R_f 0.54 (system 2) [(–)-epicatechin-3-*O*-gallate (**6**)]; glucose (**4**), R_f 0.51 (PC, system 6); and a compound with mp 135–137°C, $[\alpha]_D^{24}$ –26.5° (*c* 0.21, acetone) [galloylglucose (**9**)].

Thiolytic Cleavage of Proanthocyanidins. Compound **I** (200 mg) and thiophenol (5 mL) were mixed, treated with acetic acid (3 mL) in EtOH (10 mL), and left at room temperature for 48 h. The course of the reaction was monitored hourly during the first 10 h by TLC. The reaction mixture was condensed. The resulting oily residue was chromatographed over Sephadex LH-20 (elution by 80% EtOH) to afford a compound (11 mg), $C_{22}H_{18}O_{10}$, mp 253–255°C, $[\alpha]_D^{20}$ –176° (*c* 0.15, MeOH), R_f 0.54 (system 2) [(–)-epicatechin-3-*O*-gallate (**6**)] and an amorphous substance (98 mg) of total thioethers.

Cleavage of Thioethers of I. Thioethers (98 mg) were mixed with EtOH:HOAc (3 mL, 9:1), treated with catalyst (Raney Ni), and held for 1 h at 50°C. Then, the reaction mixture was filtered. The filtrate was condensed and monitored using TLC to afford a compound of composition $C_{15}H_{14}O_6$, mp 241–243°C, $[\alpha]_D^{20}$ –68.4° (*c* 0.3, acetone:water, 1:1), R_f 0.57 (system 2) [(–)-epicatechin (**8**)].

Cleavage of II. Compound **II** (205 mg) was cleaved and the reaction products were purified as described above. The reaction mixture was chromatographed over Sephadex LH-20 (60% EtOH) to afford a compound (8 mg) of composition $C_{22}H_{18}O_{10}$, mp 253–255°C, $[\alpha]_D^{20}$ –176° (*c* 0.15, MeOH), R_f 0.54 (system 2) [(–)-epicatechin-3-*O*-gallate (**6**)] and a mixture of thioethers (110 mg).

Cleavage of Thioethers of II. Thioethers (110 mg) were mixed with EtOH:HOAc (4 mL, 9:1), treated with catalyst (Raney Ni), and held for 1 h at 50°C. Then, the reaction mixture was filtered. The filtrate was condensed and chromatographed over Sephadex LH-20 to afford two compounds, the first of composition $C_{22}H_{18}O_{10}$, mp 195–197°C, $[\alpha]_D^{22}$ +3.2° (*c* 0.52, acetone:water, 1:1), R_f 0.70 (system 1) [(+)-catechin-3-*O*-gallate (**12**)]; the second of composition $C_{22}H_{18}O_{10}$, mp 253–255°C, $[\alpha]_D^{20}$ –176° (*c* 0.15, MeOH), R_f 0.54 (system 2) [(–)-epicatechin-3-*O*-gallate (**6**)].

Enzymatic Hydrolysis of I and II. The glycoside (15 mg) was dissolved in water (10 mL) and treated with β-glucosidase. The reaction mixture was placed in a thermostat and held at 30°C for 6 h. Polyphenols were precipitated by lead acetate solution. Paper chromatography detected in the filtrate glucose (R_f 0.51, system 6).

REFERENCES

1. R. A. Muzychkina, D. Yu. Korul′kin, and Zh. A. Abilov, *Qualitative and Quantitative Analysis of the Principal Groups of BAC in Medicinal Plant Raw Material and Phytopreparations* [in Russian], Kazak University, Almaty, 2004.
2. Z. A. Kuliev, A. G. Kurmukov, V. M. Malikov, M. I. Aizikov, and M. R. Yagudaev, *Khim.-farm. Zh.*, No. 1, 55 (1991).
3. Z. A. Khushbaktova, V. N. Syrov, and Z. A. Kuliev, *Khim.-farm. Zh.*, **9**, 1111 (1989).
4. A. L. Molan, S. Sivakumaran, P. A. Spencer, and L. P. Meagher, *Res. Vet. Sci.*, **77**, 239 (2004).
5. A. E. Hagerman, K. M. Riedl, A. Jones, A. N. Sovik, N. T. Ritchard, P. W. Hartzfeld, and T. L. Riechel, *J. Agric. Food Chem.*, **46**, 1887 (1998).
6. S. Z. Nishanbaev, Z. A. Kuliev, N. K. Khidyrova, A. D. Vdovin, N. D. Abdullaev, and Kh. M. Shakhidoyatov, *Khim. Prir. Soedin.*, 325 (2005).
7. S. Z. Nishanbaev, Z. A. Kuliev, N. K. Khidyrova, A. D. Vdovin, and Kh. M. Shakhidoyatov, in: *International Scientific-Technical Conference "Prospects for Development of the Chemistry and Practical Application of Alicyclic Compounds"* [in Russian], Volgograd, 2008, p. 156.

8. S. Z. Nishanbaev, Z. A. Kuliev, N. K. Khidirova, A. D. Vodvin, Kh. M. Shakhidoyatov, and N. D. Abdullaev, in: *8th International Symposium on the Chemistry of Natural Compounds (8th SCNC)*, June 15-17, 2009, Eskisehir, Turkey, O-11.
9. M. I. Zaprometov, *Fundamentals of Polyphenolic Compounds* [in Russian], Nauka, Moscow, 1974, p. 63.
10. A. D. Vdovin, Z. A. Kuliev, and N. D. Abdullaev, *Khim. Prir. Soedin.*, 535 (1997).
11. Y. Kashiwada, G. Nonaka, and I. Nishioka, *Chem. Pharm. Bull.*, **34**, 4083 (1986).
12. V. K. Sethi, S. C. Taneja, K. L. Dhar, and C. K. Atal, *Phytochemistry*, **23**, 2402 (1984).
13. Z. Czochanska, L. Y. Foo, R. H. Newman, and L. J. Porter, *J. Chem. Soc., Perkin Trans 1*, 2278 (1980).
14. F. Hsu, G. Nonaka, and I. Nishioka, *Chem. Pharm. Bull.*, **33**, No. 8, 3293 (1985).
15. A. S. Shashkov and O. S. Chizhov, *Bioorg. Khim.*, **2**, 437 (1976).
16. Kim Khvan Khi, Z. A. Kuliev, A. D. Vdovin, M. R. Yagudaev, and V. M. Malikov, *Khim. Prir. Soedin.*, 771 (1991).
17. G. Nonaka, M. Muta, and I. Nishioka, *Phytochemistry*, **22**, 237 (1983).