NEW OLIGOMERIC PROANTHOCYANIDINE FROM *Ziziphus jujuba*

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Chemical and spectral data establish the structure of an oligomeric proanthocyanidine PZ-5 isolated from Ziziphus jujuba.

Key words: oligomeric, proanthocyanidine, isolation, structure.

We previously reported new compounds isolated from bark of *Ziziphus jujuba* [1]. In continuation of these studies, we isolated another oligomeric proanthocyanidine called proanthocyanide PZ-5 (**1**) from bark of this plant growing in Tashkent district during fruit ripening.

On the base of the physicochemical constants ($C_{75}H_{62}O_{31}$, mol. wt. 1458, $[\alpha]_D^{24}$ +76.5°), of chemical decomposition products, and spectroscopic data (UV, IR, ^{13}C NMR) we established the structure and relative configuration of this compound. Compound **1** gives with vanillin—H_2SO_4 a red color which is typical for proanthocyanidines.

Base hydrolysis of **1** under a N_2 atmosphere produced phloroglucinol and three aromatic acids: *p*-hydroxybenzoic, protocatechuic, and gallic. This indicates that this proanthocyanidine has a complicated composition.

Acid cleavage of **1** produces a hydrolysate that contains four compounds: pelargonidin (**2**), cyanidine (**3**), delphinidin (**4**), and (+)-catechin (**5**). Thiolytic decomposition gives four compounds: (+)-catechin from the "lower" unit and a mixture of three thioesters (**6**, **7**, and **8**) from the "upper" unit. The thioesters were reduced in weak acid over Raney nickel catalyst to give (-)-epiafzelechin (**9**), (+)-catechin (**5**), and (-)-epigallocatechin (**10**).

The ^{13}C NMR spectra (Table 1) support the assignment of the products of base cleavage, acid hydrolysis, and thiolytic decomposition of **1**.

The chemical shifts of C-2′, C-6′, and C-4′ at 129.4, 129.8, and 156.7 ppm, respectively, are consistent with the presence of the afzelechin unit. Chemical shifts typical of C-2′, C-6′, and C-4′ of the gallocatechin unit appear at 106.8 and 133.9 ppm. Resonances of C-2′, C-5′, and C-6′ of ring B and C-2 of ring C of (+)-catechin are observed at 115.6, 116.4, 119.6, and 81.3 and 83.2 ppm.

The interflavane bond in **1** is the C-4β–C-8 and C-4α–C-8 type. Signals of C-10 (101.3 ppm) confirm this.

The physicochemical properties, chemical composition, and molecular weight indicate that **1** is the pentameric proanthocyanidine (-)-epiafzelechin-(4β-8)-(-)-epigallocatechin-(4β-8)-(+)-catechin-(4α-8)-(-)-epigallocatechin-(4β-8)-(+)-catechin.

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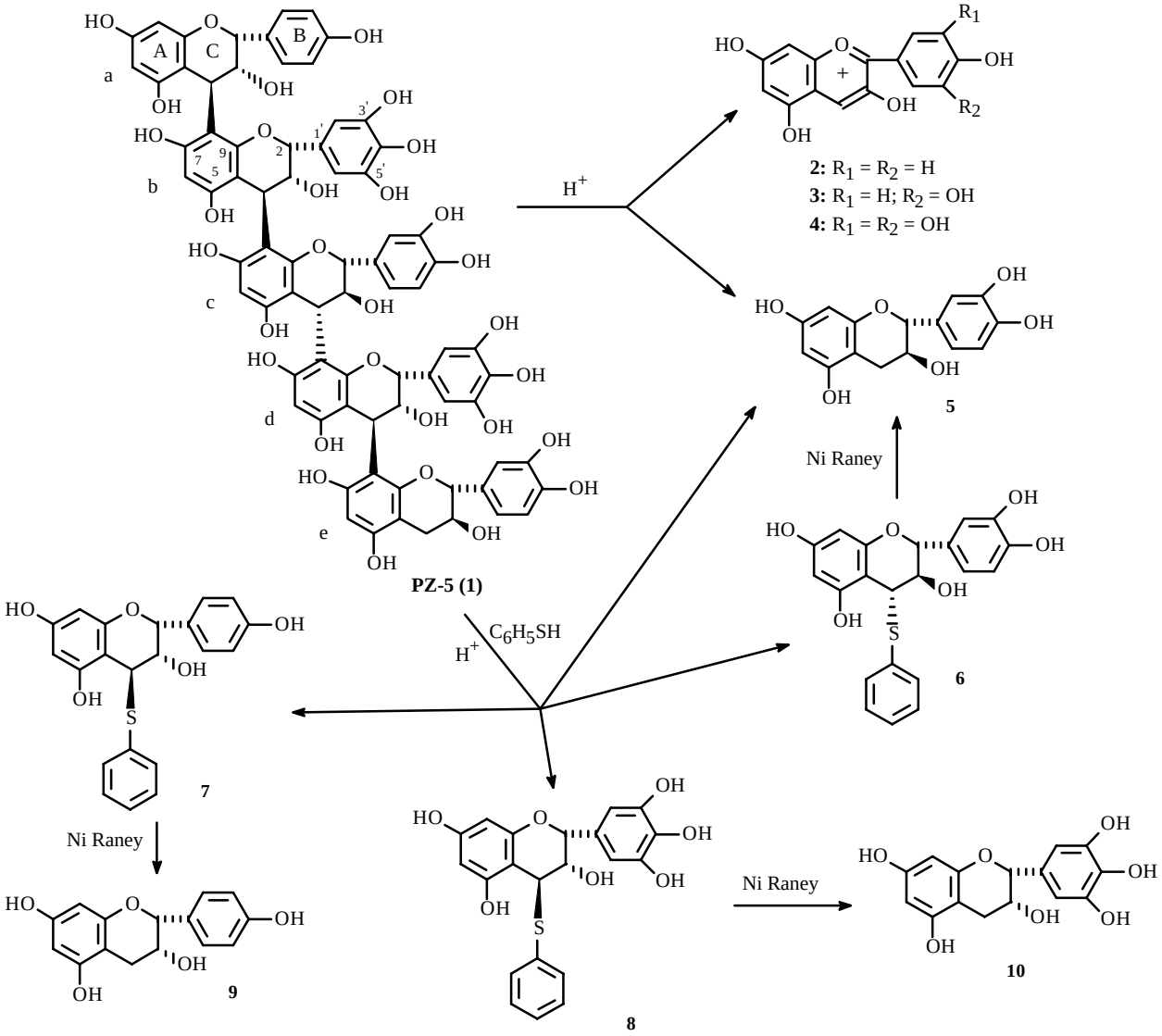
TABLE 1. Chemical Shifts (ppm) in ^{13}C NMR Spectrum of **1**

C atom	Fragment PZ-5			
	a	b, d	c	e
C-2	77.1	77.1	83.2	81.7
C-3	72.2	72.2	70.3	67.7
C-4	36.9	36.9	37.4	-*
C-6	96.7	96.0	96.0	96.0
C-8	96.4	107.4	107.4	107.4
C-10	101.3	101.3	101.3	101.3
C-5,7,9		153.6-157.8		
C-1′	131.6	130.3	131.6	131.6
C-2′	129.8	106.8	117.0	115.6
C-3′	114.6	145.4	147.3	145.1
C-4′	156.7	133.9	147.3	145.1
C-5′	114.6	145.4	117.0	116.4
C-6′	129.4	106.8	120.1	119.6

Signal overlapped by solvent signal.



EXPERIMENTAL

General. UV spectra of proanthocyanidines and their derivatives were recorded in alcohol solution on a Perkin—Elmer-Lambda-16 instrument; IR spectra, on a Perkin—Elmer-2000 FT-IR instrument in KBr pellets. ^{1}H and ^{13}C NMR spectra were obtained on a Tesla BS-567A/100 (^{1}H) and 25 (^{13}C) instrument in $(CD_3)_2CO$—D_2O (1:1) solution with TMS as internal standard (δ-scale). The molecular weight was determined in a MOM 3170 ultracentrifuge and by gel filtration over a calibrated column of Sephadex LH-20; the optical activity, on a JASCO-J-20 instrument. The purity of the compounds was determined using paper chromatography and TLC over Silufol UV-254 plates [2] using vanillin (1%) in alcoholic H_2SO_4 (5-10%) as developer.

Extraction and Isolation of Proanthocyanidines. Bark of *Z. jujuba* (5.8 kg) was extracted six times with ethanol (80%). The extracts were combined and evaporated in vacuo at 40°C to 2 L. The condensed extract was treated successively with diethylether, ethylacetate, and *n*-butanol to give extracts of 18.2, 21.1, and 165 g, respectively. High-molecular-weight proanthocyanidines (510 g) were isolated from the aqueous remainder.

Separation of Proanthocyanidines. The butanol extract (85 g) was mixed with cellulose (85 g), placed on a column of microcrystalline cellulose (800 g), and eluted by $CHCl_3$—ethylacetate (1:10-1:20), ethylacetate, and acetone to give fractions of 100 mL. The elution was monitored using TLC. Eluates 80-196 contained a mixture of relatively low-molecular-weight proanthocyanidines, were combined, evaporated, and rechromatographed (41.9 g) over cellulose with elution by ethylacetate and ethylacetate—acetone (10:1-1:1).

Compound 1. Solid from fractions 131-158 (1.325 g) was chromatographed over Sephadex LH-20 with elution by ethanol (60%). Yield 1.213 g of amorphous substance, $C_{75}H_{62}O_{31}$, mol. wt. 1458, $[\alpha]_D^{24}$ +76.5° (*c* 1.0, acetone—water, 1:1). Table 1 contains the ^{13}C NMR data.

Base Cleavage of 1. Compound **1** (50 mg) was cleaved by the literature method [3]. Phloroglucinol, *p*-hydroxybenzoic, protocatechuic, and gallic acids were detected and identified.

Acid Cleavage of 1. Compound **1** (150 mg) produced (+)-catechin {mp 178-179°C, $[\alpha]_D^{24}$ +18.3° (*c* 0.9, acetone—water, 1:1)} and the anthocyanidines: cyanidin [R_f 0.69 (2 N HCl), λ_{max} 552 (0.1% HCl in ethanol)], delphinidin [R_f 0.36 (2 N HCl), λ_{max} 554 nm (0.1% HCl in ethanol)], and pelargonidin [R_f 0.80 (2 N HCl), λ_{max} 518 nm (0.1% HCl in ethanol)].

Thiolytic Cleavage of 1. Compound **1** (350 mg) was cleaved and the products purified as before [1] to give (+)-catechin (7 mg) and a mixture of thioesters (259 mg). The thioesters (200 mg) were mixed with ethanol—acetic acid (9:1, 12 mL) and treated with Raney nickel catalyst at 50°C for 1 h. The reaction mixture was filtered. The filtrate was condensed and chromatographed over Sephadex LH-20 with elution by ethanol (80%) to give (-)-epiafzelechin {mp 249-250°C, $[\alpha]_D^{22}$ -52° (*c* 1.0, acetone)}, (-)-epigallocatechin (mp 241-243°C, $[\alpha]_D^{24}$ -69°), and (+)-catechin.

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