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DIMERIC PROANTHOCYANIDINS OF *Hibiscus cannabinus*

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Two dimeric proanthocyanidins have been isolated from the bark and roots of kenaf of variety Uzbekskii 1574. On the basis of chemical transformations and NMR and mass-spectral analyses it has been established that the proanthocyanidins have the structure of a dehydro dimer of 3,3',4',5,7-pentahydroxyflavan and that of 8-(3-galloyloxy-3',4',5,7-tetrahydroxyflavan-4-yl)-3,3',4',5,7-pentahydroxyflavan, respectively, with a C_4—C_8 (or C_6) bond between the flavan moieties.

In the bark of the roots of *H. cannabinus* L. (kenaf) of the variety Uzbekskii 1574 (collected in the "Politotdel" kolkhoz [collective farm], Tashkent province), in addition to monomeric flavan-3-ols, two dimeric proanthocyanidins have been detected.

The previously deresinified and defatted bark of the kenaf roots was steeped with methanol. From the concentrated methanolic extract by treatment with ether and ethyl acetate and then by column chromatography on silica gel and polyamide two proanthocyanidins were isolated which we have called B5 and B6; on being heated with 2 N HCl they formed the anthocyanidin pigment cyanidin.

B5 is an amorphous substance giving an acetyl derivative melting at 167-169°C. From the results of chemical transformations and UV, IR, and NMR spectroscopy, B5 has the structure of a dehydro dimer of 3,3',4',5,7-pentahydroxyglavan with a C_4—C_8 (or C_6) bond between the flavan moieties with the trans configuration (2R:3S) of the asymmetric centers of the "upper" and the cis configuration of the "lower" half of the molecule; it was identical with the dimeric proanthocyanidin 4 isolated previously from the stems of *Cotoneaster oligantha* Pojark [1]. This is the first time that it has been isolated from *H. cannabinus*.

B6 is an amorphous cream-colored substance very labile and having no clear melting point but beginning to soften at 155°C; $[\alpha]_D^{20}$ +144.0° (c 0.24; ethanol).

Under the action of 0.1 N HCl, B6 formed a pigment and catechins, which were isolated and identified as cyanidin (—)-epicatechin gallate and (—)-epicatechin by comparison with authentic samples on chromatograms and from the products of alkaline degradation. Under the action of 0.01 N HCl under mild conditions, B6 was hydrolyzed with the formation of (—)-epicatechin gallate and (—)-epicatechin. When sulfur dioxide was passed through B6, (—)-epicatechin gallate and (—)-epicatechin were formed.

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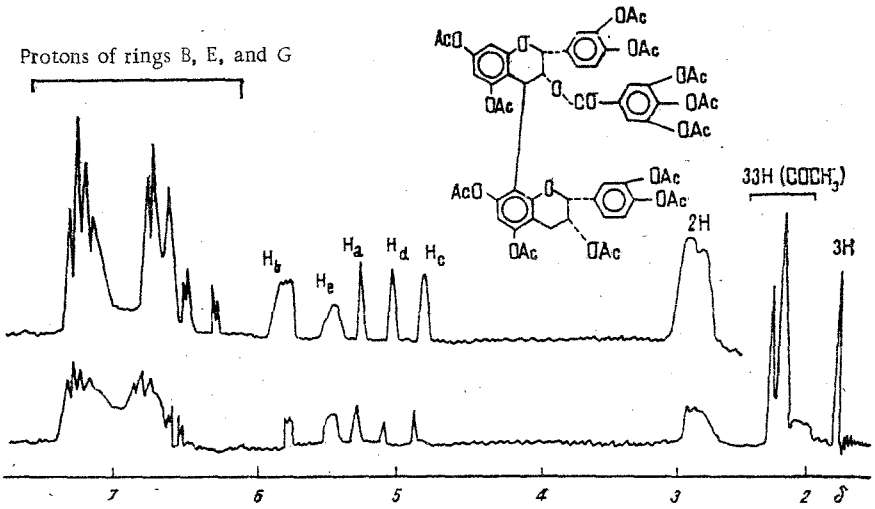


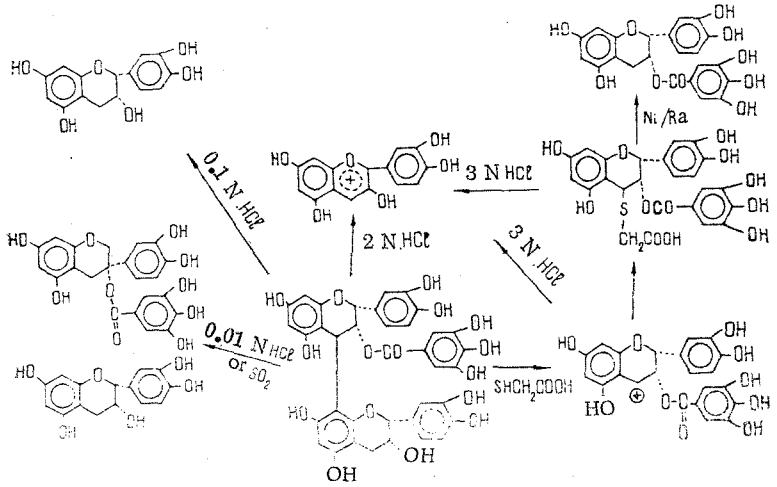
Fig. 1. NMR spectrum of the acetyl derivative of the dimeric proanthocyanidin B6 (in $CDCl_3$).

The results of acid degradations show that both the "upper" and "lower" halves of the molecule have the 2R:3R configuration but that they differ in their degrees of hydroxylation.

To study the "upper" and "lower" halves of the dimer, and also the position of formation of the molecular bond, B6 was cleaved under the action of thioglycolic acid at room temperature [2]. The process was monitored by PC in systems 1 and 3. After only 10 min, the action of thioglycolic acid formed (−)-epicatechin and a flavan thioester which were detected on chromatograms with a vanillin reagent. With this reagent, thioesters give a yellow coloration which rapidly disappears. By diluting the reaction mixture, the thioester was isolated in the form of a cream-colored oil with R_f 0.81 in system 1.

On being heated with 2 N HCl, the thio derivative was converted into cyanidin. Reduction of the thioester on Raney nickel yielded (−)-epicatechin gallate [3].

The capacity for being cleaved under the action of thioglycolic acid in mild conditions with the formation of a catechin and a thioester shows that this proanthocyanidin is dimeric with a labile C_4—C_8 (or C_6) bond between the flavan units, its "upper" half being represented by (−)-epicatechin gallate and its "lower" half by (−)-epicatechin (scheme).



The chemical study of the dimeric proanthocyanidin B6.

The acetylation of B6 gave a dodecaacetate in the form of a white substance with mp 155-158°C (R_f 0.15 in system 4).

The methylation of B6 with diazomethane yielded an amorphous substance the subsequent acetylation of which gave a monoacetyl undecamethyl derivative showing that the hydroxyls of B6 do not participate in the formation of the interflavan bond.

The NMR spectrum of the acetyl derivative, taken in deuterated chloroform in acetone (Fig. 1), shows the signals of 33 aromatic protons at δ 2.2-2.3 ppm and the signals of three aliphatic protons at δ 1.82 ppm, confirming that all 12 hydroxy groups of the proanthocyanidin undergo acetylation and do not participate in the formation of the interflavan bond.

At δ 7.28-7.30 ppm there are the signals of protons of ring G, and at δ 7.30-7.35 ppm all six protons of rings B and E. At δ 6.25-6.45 ppm there are the signals of the protons of rings A and D in the form of a doublet (J = 2 Hz), which characterizes a meta interaction of protons.

A one-proton singlet at 6.75 ppm shows the disappearance of a proton from the phloroglucinol ring and therefore the participation of the C_8 or C_6 position of the flavanoid unit in the interflavan bond of the dimer. At δ 2.79 ppm in the spectrum of the dodecaacetate of B6, the two protons of a methylene group form a broadened doublet with $J_{3,4}$ = 2 Hz, which is characteristic for interaction with one equatorial proton. The presence of only two methylene protons and not four shows the participation of C_4 of one of the flavan units in the bond.

The H_e proton gives a signal in the form of a singlet at δ 5.1 ppm, and the H_a proton one at δ 5.30 ppm. The absence of a splitting of the signals of these protons shows the cis position of the C_2 and C_3 protons of both halves of the dimer.

The protons of the heterocyclic rings H_b and H_e resonate at δ 5.85 and 5.38 ppm. The H_c proton of the "upper" half of the molecule is represented by a signal at 4.70 ppm in the form of a doublet with $J_{3,4}$ = 1 Hz. In this case, this constant does not permit an unambiguous answer to the question of the configuration of the C_4 proton, since in both the axial and equatorial positions of the proton interaction with the equatorial C_3—H leads to a small SSCC ($J_{3,4}$).

A mass-spectrometric analysis of the monoacetylundecamethyl derivative of B6 showed that the fragmentation takes place by the RDA route with the simultaneous elimination of acetic acid and methyl gallate, and the diflavene formed breaks down into two flavylium ions with the same mass, 327 m/z, which is in complete agreement with literature information on the lability of a C_4—C_8 (or C_6) bond in the dimeric proanthocyanidins of group B [4-6].

Thus, the results of chemical investigations and of NMR and mass spectroscopy showed that proanthocyanidin B6 has the structure of the dimer 8-(3-galloyloxy-3',4',5,7-tetrahydroxyflavan-4-yl)-3,3',4',5,7-pentahydroxyflavan. This compound is a new one not previously described in the literature and this is the first time that it has been isolated from *Hibiscus cannibinus*.

EXPERIMENTAL

Solvent systems for PC: 1) butan-1-ol—acetic acid—water (40:12:28); 2) acetic acid—hydrochloric acid—water (5:1:6); 3) 2% acetic acid; and 4) benzene—acetone (8:2). Angles of rotation were determined on a SM circular polarimeter. NMR spectra were recorded on a Varian XL-100 instrument in deuterated chloroform and acetone, the chemical shifts being given in the δ scale, and mass spectra on a MKh-1303 instrument. Silica gel, polyamide, Chromaton, Fin No. 11 chromatographic paper (GDR), and Silufol UV-254 (Czechoslovakia) were used as adsorbents. The results of elementary analysis agreed with the calculated figures.

Isolation of the Dimeric Proanthocyanogens. The air-dry bark of the roots of kenaf of variety Uzbekskii 1574 collected after the withering of the phloem of the green part (3 kg) was comminuted and treated successively with benzene and chloroform for a week (three times each) to eliminate fat and resins. Then the raw material was steeped with methanol (extract 1) and with 70% aqueous methanol (extract 2). After evaporation, the extracts were combined, water was added and the mixtures were extracted with ether and ethyl acetate until the reaction with vanillin was negative. The ethyl acetate fraction mainly contained dimeric proanthocyanidins and catechins. The substances of the ethyl acetate fraction were separated by adsorption chromatography from silica gel and polyamide. The columns were eluted with ether and with a mixture of ether and ethyl acetate. The fractions containing the proanthocyanidins were passed through polyamide again, being eluted with mixtures of chloroform and methanol with a gradient increase in the amount of the latter. Two dimeric proanthocyanidines, B5 and B6, were obtained (0.271 g and 0.252 g, respectively).

Conversion into Anthocyanidin. A 5-mg sample of each proanthocyanidin in 2 ml of 2 N HCl in methanol was heated on the boiling water bath for 20 min, the reaction mixture becoming red. The pigment was extracted with isoamyl alcohol. It was analyzed by PC in system 2 with known anthocyanidins as markers. Only cyanidin with Rf 0.33, λ_{max} 275, 535 nm was obtained.

Acid Hydrolysis of Proanthocyanidin B6. A mixture of 10 mg of proanthocyanidin B6 and 2 ml of 0.1 N HCl in methanol was heated on the water bath. The reaction was monitored by PC with markers every 5 min for an hour. For the first thirty min, (−)-epicatechin and (−)-epicatechin gallate were detected in the degradation products. On further heating cyanidin was detected in addition to the catechins. Under the action of 0.01 N HCl, only the catechins were detected.

Cleavage of B6 with Thioglycolic Acid. A solution of 20 mg of proanthocyanidin B6 in 3 ml of ethanol was treated with 3 ml of thioglycolic acid. The reaction was carried out at room temperature for 24 h. Samples were taken after 30 min and 1, 2, 3, 4, 5, 8, and 24 h. The reaction products were analyzed by unidimensional PC with markers in system 1. (−)-Epicatechin and a flavan thioester were obtained.

Isolation of the Thioester. A saturated solution of sodium bicarbonate was added to the reaction mixture, and it was extracted with ethyl acetate. (−)-Epicatechin was detected. The aqueous part was acidified and was then likewise extracted with ethyl acetate, and after the ethyl acetate had been distilled off thio derivatives of the proanthocyanidin were obtained in the form of an oil with Rf 0.81 in system 4.

Reduction of the Thio Derivatives. To 5 mg of the thioester was added 5 ml of a suspension of Ni/Ra in ethanol and the mixture was left at room temperature for 2 h. Then the catalyst was filtered off and the reduction products were analyzed by PC with catechin markers in systems 1 and 3. (−)-Epicatechin gallate was detected in the reduction products of the proanthocyanidin.

Transformation of the Thioester into Anthocyanidin. The thioester (3 mg) was treated with 2.5 ml of a 3 N solution of HCl in methanol, and the mixture was heated on the water bath for 30 min, becoming red. After the addition of water, it was extracted with butan-1-ol. Cyanidin was identified by PC of the butanol solution in system 2.

Acetylation of the Dimeric Proanthocyanidin B6. The acylation of 10 mg of the dimer dried over P_2O_5 in vacuum was carried out in the usual way. The precipitate that deposited was separated off and was washed with water to eliminate pyridine. It was purified by column chromatography with Chromaton–silica gel (5:1) as the adsorbent. Elution was performed with acetone–benzene (2:8). This gave the dodecaacetate of the dimer in the form of a white substance with mp 155-158°C, Rf 0.15 in system 4.

Methylation of Proanthocyanidin B6. In the form of a solution in 2 ml of ethanol, 50 g of the dimeric proanthocyanidin was methylated four times successively with diazomethane for 48 h. Each time, the reaction mixture, after being poured into ice water, was left at 5°C for 5 h. The precipitate that deposited was filtered off and washed with cold water and was recrystallized from methanol. This gave the undecamethyl ether of the dimer B6, which was then acetylated by the usual method, leading to the acetylundecamethyl derivative in the form of an amorphous white substance with Rf 0.13 in system 4.

SUMMARY

Two dimeric proanthocyanidins have been isolated from the bark of the roots of *H. cannabinus* and their structures have been established on the basis of chemical transformations, NMR analysis, and mass spectra.

Proanthocyanidin B5 has the structure of a dehydro dimer of 3,3',4',5,7-pentahydroxyflavan with a C_4–C_8 (or C_6) bond between the flavan molecules and the trans configuration (2R:3S) configuration of the asymmetric centers of the "upper" half and the cis configuration of the "lower" half of the molecule.

Proanthocyanidin B6 has the structure of 8-(3-galloyloxy-3',4',5,7-tetrahydroxyflavan-4-yl)-3,3',4',5,7-pentahydroxyflavan and the cis configuration (2R:3R) of the asymmetric centers of both halves of the molecule.

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STRUCTURE AND CONFIGURATION OF STENANZINE

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From the total alkaloids isolated by chloroform extraction of the epigeal part of *Rhinopetalum stenantherum* a new base has been isolated — stenanzine — with mp 203-205°C, $[\alpha]_D$ −44°, $C_{27}H_{43}NO_3$. On the basis of a study of the IR, NMR, and mass spectra of stenanzine and its conversion products the configuration and structure of 3β,23α-dihydroxy-5α-veratr-12-enin-6-one have been established for this alkaloid.

From the combined alkaloids obtained by the chloroform extraction of the epigeal part of *Rhinopetalum stenantherum* Rgl. [1, 2], by chromatography on a column of alumina, a new alkaloid stenanzine with mp 203-205°C $[\alpha]_D$ −44° $C_{27}H_{43}NO_3$, (I), has been isolated.

The IR spectrum of (I) shows absorption bands at (cm^{-1}) 3425-3125 (—OH, NH—), 2930-2830, 1475, 1455, 1420 ($-CH_3$; $-CH_2-$); 1713 (C=O). The mass-spectrometric fragmentation of stenanzine took place similarly to that of veratramine: peaks were observed with m/z 96, 114 (100%), 115, 141, 256, 315, $(M-1)^+$, 429 M^+ [3-5].

The acetylation of stenanzine with acetic anhydride in pyridine yielded 0,0',N-triacetylstenanzine (II), the IR spectrum of which had absorption bands at (cm^{-1}) 1740, 1245 (C=O, ester); 1717 (C=O), and 1648 ($N-COCH_3$) and no absorption bands of hydroxy groups. When 0,0',-N-triacetylstenanzine was saponified in a methanolic solution of caustic soda, N-acetylstenanzine (III) was obtained with M^+ 471. Its IR spectrum contained absorption bands at (cm^{-1}) 3450 (OH); 2970-2860, 1455, 1430 ($-CH_3$; $-CH_2-$); 1717 (C=O); and 1595 ($N-COCH_3$), and the absorption bands of the ester carbonyl group had disappeared.

The reduction of stenanzine with sodium tetrahydroborate led to a dihydro derivative $C_{27}H_{45}NO_3$ (IV), M^+ 431. Details of the NMR spectra of (I) and (II) are given in Table 1.

A comparison of the NMR and mass spectra of stenanzine and of peimisine (V) (see Table) [3] shows that stenanzine belongs to the C-nor,D-homosteroid alkaloids of the jervine group [3-5]. The NMR spectrum of (I) shows the signals from two protons geminal to hydroxy groups at 3.76 ppm (br.s, $W_{1/2}$ = 6 Hz) and 3.65 ppm ($W_{1/2}$ = 22 Hz). Consequently, both hydroxy groups have a secondary nature, as was confirmed by the production of 0,0',N-triacetylstenanzine. In the mass spectrum of (I), together with the peak of the molecular ion with m/z 429, the peak of an ion with m/z 114 (100%) is also observed, which shows the position of one of the hydroxy groups in ring F [4, 5]. The hydroxy group may occupy one of the two possible positions at C_{23} and C_{24}. Of these, from biogenetic considerations, the position at C_{23} is most suitable.

The position of the other secondary hydroxy group and of the carbonyl group was determined by comparing the chemical shifts of the 19-CH_3 group of stenanzine (I) and its acetate with those of the 19-CH_3 groups in the spectra of peimisine (V) and its acetyl derivative

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