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PROANTHOCYANIDIN DIMERS FROM *Spiraea hypericifolia*

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UDC 547.972

Continuing a study of the flavans of individual parts of *Spiraea hypericifolia* L., we have detected the presence in the bark and roots of this plant of three dimeric flavans and have established their structures as (—)-epicatechin-(+)-catechin (flavan I) and (—)-epicatechin-(—)-epicatechin (flavan II).

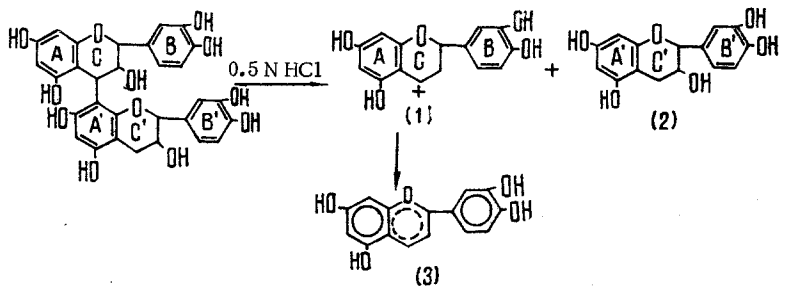
Like all flavans, the substances investigated give a red coloration with vanillin in concentrated HCl [1] and an insoluble phlobaphene on being heated with dilute mineral acids [2]. The alkaline cleavage of each of the compounds formed phloroglucinol and protocatechuic acid.

Heating the substances with 2 N HCl in methanol led to the formation of cyanidin, which was identified by paper chromatography in the solvent system hydrochloric acid—acetic acid—water (5:1:5) and by UV spectroscopy (λ_{max} 535 nm in ethanol). The substances differed in the products of their acid cleavage under mild conditions (0.05 N HCl): the flavan (I) formed (+)-catechin and traces of (—)-epicatechin and flavin (II) formed (—)-epicatechin.

Cleavage under mild conditions in an acid medium gave the catechin from the "bottom" part of the molecule in view of the fact that the conjugated orientation of the hydroxy

S. M. Kirov Kazakh State University, Alma-Ata. Translated from Khimiya Prirodnykh Soedinenii, No. 6, pp. 735-742, November-December, 1976. Original article submitted July 29, 1976.

groups in ring A favors the formation of nucleophilic centers in the C_6 and C_8 positions. Under the action of the proton of an acid, the C_4—C_8 bond weakens, which leads to heterogeneous decomposition — to the formation from the "top" part of the molecule of a monomeric carbocation (1) and from the "bottom" part of catechin (2). Then the carbocation (1) in a more acid medium (2 N HCl) is converted by oxidation into the flavylium cation (3):



The ready splitting off of catechin on acid hydrolysis of the flavans (I) and (II) with dilute acid and the formation of cyanidin under the action of more concentrated acid shows that the flavans are proanthocyanidin dimers with a C_4—C_8 bond between the flavan units [3, 4].

Flavans (I) and (II) formed amorphous white powders of the same composition, $C_{30}H_{26}O_{12}$. Flavan I had R_f 0.22 in the butan-1-ol—acetic acid—water system (system 1) and 0.50 in 2% acetic acid (system 2), and flavan (II) had R_f 0.50 and 0.55 in systems 1 and 2, respectively. The substances were very labile, rapidly becoming pink on storage, and therefore we studied their decaacetyl derivatives, obtained by acylation of the corresponding flavans (I and II). The acetyl derivatives (III and IV) had the same composition, $C_{50}H_{46}O_{22}$.

Derivative (III) had mp 133-135°C, $[\alpha]_D^{20}$ +85.5° (c 0.7; ethanol), R_f 0.43 on TLC in the benzene—acetone (8:2) system (system 3), and derivative IV had mp 128-130°C, $[\alpha]_D^{20}$ +44.0° (c 0.32; chloroform), R_f 0.30 on TLC in system 3. The NMR spectra of the decaacetyl derivatives of the flavans (Fig. 1), taken in $CDCl_3$ and CCl_4, contained the signals of 24 protons of aromatic acetyl groups at δ 2.15-2.32 ppm (substance III) and 2.18-2.32 ppm (substance IV), and also the signals of six protons of aliphatic acetyl groups at δ 1.78-1.82 ppm in (III) and 1.82 ppm in (IV). The presence of 10 acetyl groups in both substances shows that all the hydroxy groups of the units (catechins) forming the flavans are free and do not participate in the formation of the bond between the flavan units.

The spectra of the derivatives of both flavans show the signals of the six protons of rings B and B' at δ 6.80-7.24 and 6.84-7.40 ppm, respectively. In the spectrum of compound (III), the signals of the protons of rings A and A' give two one-proton doublets at δ 5.88 and 6.20 ppm with J = 2 Hz, and in the spectrum of (IV) the corresponding signals appear at δ 5.92 and 6.18 ppm, again with J = 2 Hz. These splitting constants are characteristic for meta-substituted rings. A singlet at δ 6.50 ppm with an integral intensity of one proton relates to proton 6 of ring A' of compound (III), and a singlet at 6.55 ppm to proton 6 of ring A' of compound (IV). The absence of a signal from H-8' of ring A' in both compounds is confirmed by the shape of the signal (singlet) of H-6 of ring A and shows the participation of the C_8 carbon atom in the bond between the flavan units.

In the spectrum of compound (III), the protons of the heterocyclic rings C and C' are represented by the signals of the C-2 and C'-2 protons at δ 5.42 and 4.34 ppm, and of C-3 and C'-3 at 5.06 and 5.12 ppm, respectively, and also those of one methylene group — two one-proton multiplets at 2.5 and 3.1 ppm and one H-4 at 4.33 ppm. The absence of a signal of one of the protons at C_4 shows the participation of this carbon atom in the interflavan bond.

In the spectrum of compound (IV), the protons of the heterocyclic rings are represented by the signals of C-2 and C'-2, the protons at δ 5.58 and 4.62 ppm, of the C-3 and C'-3 protons at 5.12 ppm, by the H-4 signal at 4.42 ppm, and by the signal of the protons of one methylene group — a two-proton doublet at 2.88 ppm.

Thus, the absence of a proton at C-4 of one C ring and of a proton at C-8' of ring A' shows a C_4—$C_{8'}$ bond between the flavan units.

The dimeric structure and the C_4—$C_{8'}$ form of the bond were confirmed by the mass spectrum of the octamethyl-diacetyl derivative of the flavan (II). The mass number M^+ 774 cor-

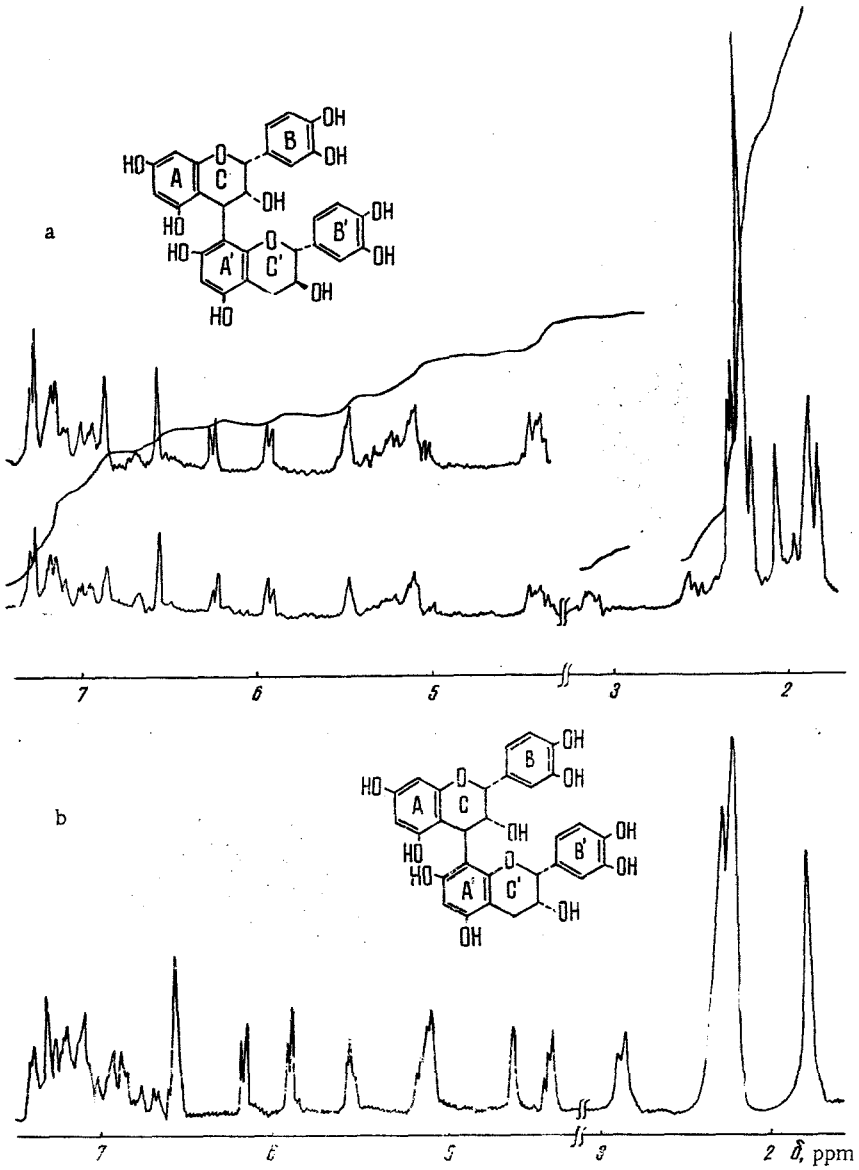
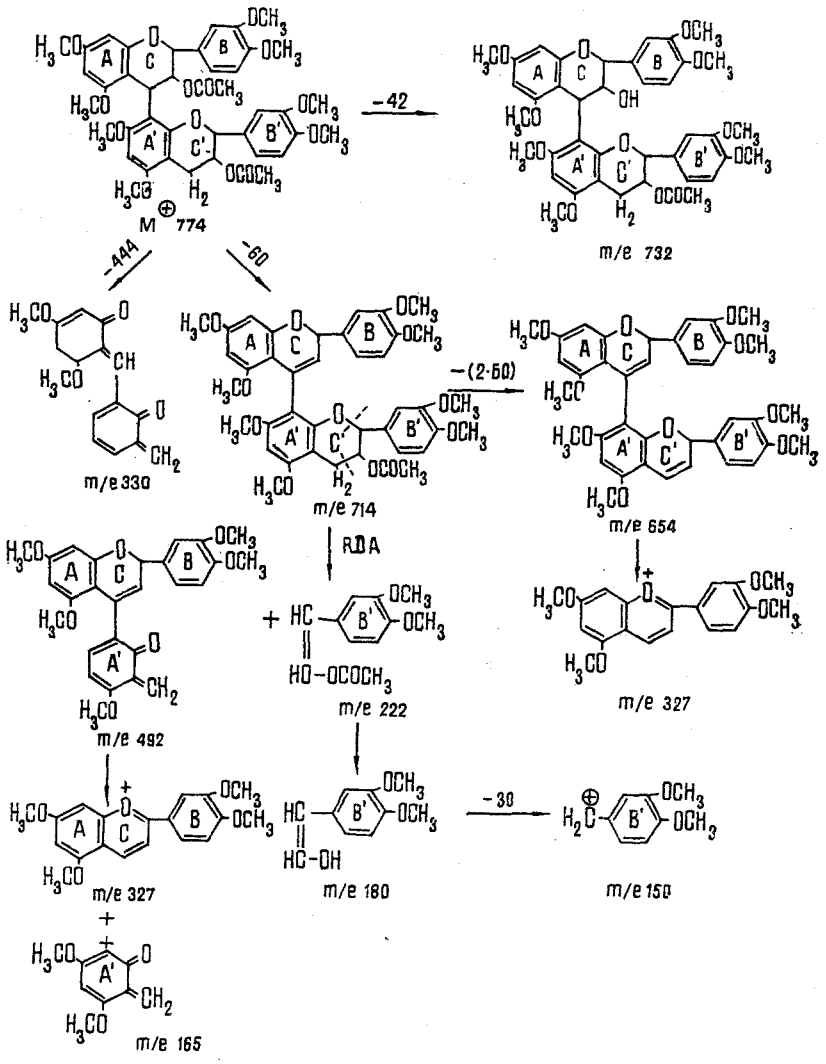


Fig. 1. NMR spectra of the decaacetyl derivatives of the flavans (I) (a) and (II) (b).

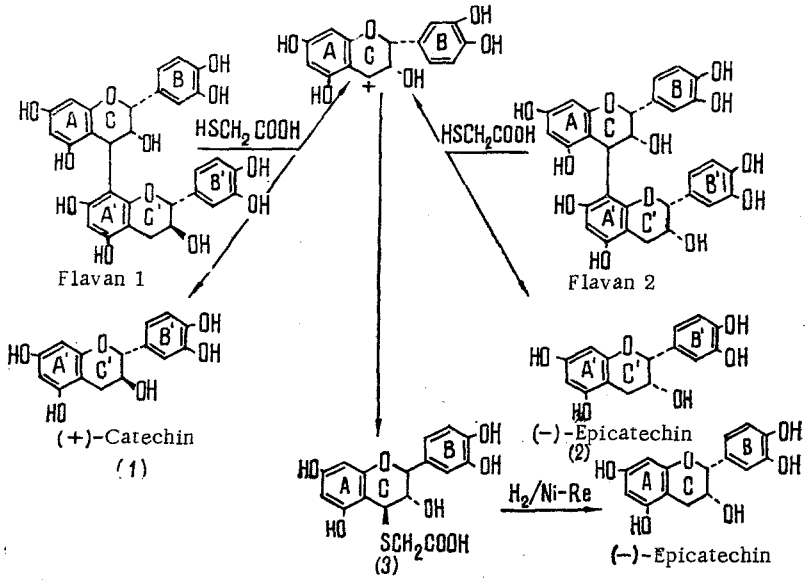
responds to the calculated molecular weight. Under electron impact, two molecules of acetic acid are split off with the formation of a flavene having m/e 714, and then a diflavene with m/e 654 (Scheme 1). The ion with m/e 714 undergoes a retrodiene cleavage, forming a fragment with m/e 492 which decomposes further to fragments with m/e 327 and 165, showing cleavage of the C_4—C_8' bond. The mass spectrum of this compound is in harmony with the literature information [5].

To determine the configurations of both flaven units ("top" and "bottom") forming the dimers we used the specific method of cleavage with thioglycolic acid [6] (Scheme 2). Thus, the action of thioglycolic acid on flaven (I) formed from its "bottom" half (+)-catechin (1), showing that its configuration was 2R,3S. Under the same conditions, the "bottom" half of the molecule of flavan (II) formed (—)-epicatechin (2), showing its configuration as 2R,3R. The cations formed from the "top" halves of the flavan molecules (I) and (II) gave the sulfide (3). After reduction of the sulfide formed on a catalyst (Raney nickel), the "top" flavan unit was found, consisting of (—)-epicatechin in both cases, showing the 2R,3R configuration of both "top" halves of the dimer molecules.

The results of a consideration of the spin—spin coupling constants of the protons of the heterocyclic rings confirmed the configuration and conformations of the asymmetric centers at the C-2 and C-3 carbon atoms found by acid hydrolysis. Thus, in the spectrum of the



Scheme 1. Fragmentation of the octamethyl-diacetyl derivative of the flavan (II).



Scheme 2. Cleavage of the proanthocyanidin dimers with thioglycolic acid.

decaacetyl derivative of the flavan (I), a broadened singlet corresponding to H-2 and H-3 of the "top" half of the molecule confirmed the cisoid (a,e) conformation of these asymmetric centers (2R,3R), corresponding to (−)-epicatechin. A doublet with $J_{2,3}$ = 10 Hz from H-2' and a multiplet due to H-3' of the "bottom" half of the dimer shows the transoid (a,a) conformation of these protons (2'R,3'S), which is characteristic for (+)-catechin. Proanthocyanidin dimers with this configuration of the asymmetric centers had been arbitrarily denoted at B-1 by Weinges [3]. For proanthocyanidins in which the "top" half of the molecule has the 2R,3R configuration it is impossible to draw an unambiguous conclusion concerning the absolute configuration of the C-4 atom, since the experimentally observed small coupling constant $J_{3,4}$ = 2 Hz shows the syn position of the protons, which is common to both the R and the S configurations. Proanthocyanidin dimers with this configuration and with a specific rotation of the decaacetyl derivative of $[\alpha]_D^{20}$ + 71.0 (c 2.0; acetone) have been isolated previously only from honeylocust fruit [7].

In the spectrum of the decaacetyl derivative of the flavan (II), broadened singlets corresponding to H-2' of the "bottom" and H-2 of the "top" halves of the molecule show their cisoid (a,e) positions with respect to C_3—H and $C_{3''}$—H. The cis form of the "bottom" half of the molecule is also shown by a two-proton doublet of a methylene group at 2.88 ppm. Consequently, both halves of the molecule have the 2R,3R configuration. Proanthocyanidin dimers with such configuration of the asymmetric centers have been provisionally denoted by Weinges as B-2 [3].

EXPERIMENTAL

The NMR spectra were taken on a Varian HA-100D spectrometer in $CDCl_3$ and CCl_4 (HMDS as internal standard). The melting points were determined on a Kofler block and the specific rotations on a CM circular polarimeter. The purity of the substances was checked by PC on FN-4 paper in the systems 1) butan-1-ol—acetic acid—water (40:12.5:29) and 2) 2% acetic acid, and by TLC on Silufol UV-254 plates in system 3) benzene—acetone (8:2). The elementary analyses corresponded to the calculated figures.

Separation of the Flavans. The comminuted bark of *Spiraea hypericifolia* was treated with benzene to remove resins and the flavonoids were extracted with methanol. From the aqueous residue after the methanol had been distilled off the monomeric flavans were extracted with ethanol and then the oligomeric flavans with ethyl acetate. The dry concentrate after the elimination of the ethyl acetate was dissolved in methanol, the solution was mixed with a small amount of Kapron, and, after drying, this was deposited on a column of polyamide. On elution with methanol—chloroform (1:1) the monomeric flavans were desorbed, and then methanol eluted substance B-1.

A methanolic extract from the roots of the same plant was treated similarly. The eluent in chromatography on polyamide was a gradient chloroform—methanol system with increasing concentrations of methanol (from 20 to 50%). The mixture containing 20% of methanol eluted the monomeric flavans, and 50% methanol eluted substance B-2. When each of the fractions was rechromatographed under the same conditions the substances were isolated in the individual state in the form of white amorphous powders. Flavan B-1 had R_f 0.22 in system 1 and 0.50 in system 2, and flavan B-2 had 0.50 and 0.55, respectively.

Alkaline Cleavage. A mixture of 5 mg of a proanthocyanidin and 1 ml of 50% KOH solution was heated at 170°C in a current of nitrogen for 20 min. The reaction mixture was neutralized with 25% sulfuric acid and extracted with ethyl acetate. The cleavage products of each of the substances investigated were shown to contain phloroglucinol and protocatechuic acid, identified with markers in solvent system 1) and also in the benzene—acetic acid—water (6:7:3) system (system 4) and the sodium formate—formic acid—water (10:200:1) system (system 5): R_f 0.72 for phloroglucinol in system 1, and R_f 0.09 for protocatechuic acid in system 4 and 0.55 in system 5.

Mild Acid Hydrolysis. To 1-2 mg of the flavan under investigation (B-1 or B-2) was added 2 ml of 0.1 N HCl, and the mixture was left at room temperature for 24 h. Samples were taken after 1, 2, 4, 6, 8, and 24 h. The hydrolyzate was analyzed by PC in systems 1 and 2 with (+)-catechin and (−)-epicatechin markers. The products of hydrolysis of flavan B-1 were found after only 2 h to contain (+)-catechin, R_f 0.66 in system 1 and 0.34 in system 2, and after 24 h there were, in addition, traces of (−)-epicatechin with R_f 0.58 in system 1 and 0.30 in system 2. (−)-Epicatechin was found in the products of the 24-hour hydrolysis of flavan B-2.

Anthocyanidin Test. The substance under investigation (1-2 mg) was dissolved in 0.5 ml of methanol, 1 ml of 2 N HCl was added, and the mixture was boiled under reflux for 15-20 min. Then it was diluted with 2-3 ml of distilled water and was shaken with isoamyl alcohol. The isoamyl alcohol extract was chromatographed in the acetic acid–hydrochloric acid–water (5:1:5) system in the presence of anthocyanidin markers. The substance responsible for the crimson color of the isoamyl alcohol, for both of the substances investigated, proved to be identical with cyanidin (R_f 0.37 in the solvent system mentioned above and λ_{max} 535 nm in ethanol).

Cleavage with Thioglycolic Acid. A solution of 2 mg of a proanthocyanidin (B-1 or B-2) in 2 ml of ethanol was heated in the boiling-water bath with 1 ml of thioglycolic acid in a current of nitrogen. Samples were taken after 1, 2, 4, 8, and 24 h and were analyzed by PC in systems 1 and 2 with (+)-catechin and (–)-epicatechin markers. After 4 h, the formation of (+)-catechin from flavan B-1 and of (–)-epicatechin from B-2 was observed, showing the configuration of the "bottom" flavan unit of the corresponding dimeric forms. The reaction mixture was treated with 3 ml of a suspension of Raney nickel catalyst in ethanol and was kept at room temperature for 2 h. Then the catalyst was filtered off and the reduction products were analyzed by PC with (+)-catechin and (–)-epicatechin markers in the systems mentioned above. The reaction products from flavan B-1 contained (–)-epicatechin in addition to (+)-catechin, and, as before, the reaction products of flavan B-2 contained only (–)-epicatechin.

Acetylation of the Proanthocyanidins. A mixture of 20 mg of the dimers (B-1 and B-2), 1 ml of dry pyridine, and 2.5 ml of acetic anhydride was kept at room temperature for 24 h. Then the solution was poured into cooled water and the mixture was left to stand at 4°C for 5 h. The precipitate that deposited was separated off and washed with water. It was purified on a column of Chromaton–silica gel (5:1) with benzene–acetone (9:1) as the eluent.

This gave the decaacetyl derivative of flavan B-1 with the composition $C_{50}H_{46}O_{22}$, mp 133-135°C, $[\alpha]_D^{20}$ +85.5° (c 0.70; ethanol), R_f 0.43 on TLC in system 3. NMR ($CDCl_3$), δ, ppm: 2.01 (3H); 2.16 (3H); 2.16 (3H); 2.21 (12H); 2.24 (3H); 2.3 (3H) ArAc and 1.78; 1.82 (6H) AlipAc.

Similarly, B-2 yielded a decaacetyl derivative with the composition $C_{50}H_{46}O_{22}$, mp 128-130°C, $[\alpha]_D^{20}$ +44.0° (c 0.32; chloroform), R_f 0.30 on TLC in system 3. NMR (CCl_4), δ, ppm: 2.2 (24 H, ArAc) and 1.82 (6 H, AlipAc).

SUMMARY

From the bark and roots of *Spiraea hypericifolia* L. we have isolated and identified proanthocyanidins consisting of dimers of 3,3',4',5,7-pentahydroxyflavans: a dimer from the bark (B-1) with the 2R:3R configuration of the asymmetric centers of the "top" half of the molecule and 2R:3S of the "bottom" half and a dimer from the roots with the 2R:3R configurations of the asymmetric centers of both the "top" and "bottom" halves of the molecule.

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