DIMERIC FLAVANOLS OF *Juniperus sabina*. I.

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Procyanidins B_2, B_3, and B_4 have been isolated from *Juniperus sabina* for the first time. On the basis of chemical transformations and the PMR spectroscopy of their decaacetates, and absolute configurations of the asymmetric centers of each of the halves of the molecules of the dimer have been determined: 2R, 3R, 4R for the "upper" half and 2R, 3R for the "lower" half of B_2, 2R, 3S, 4S for the "upper" half and 2R, 3S, for the "lower" half of B_3; and 2R, 3S, 4S for the "upper" half and 2R, 3R for the "lower" half of B_4.

Previously [1, 2] in the stems of *Juniperus sabina* L. (savin juniper) we have detected ten flavan derivatives, two of which were identified as (+)-catechin and (−)-epicatechin. In the present paper we report the isolation and study of three dimeric flavanols of group B [2].

All three flavans (I-III) were white amorphous substances with the same composition $C_{30}H_{26}O_{12}$. Under the action of alkali they decomposed into phloroglucinol and protocatechuic acid.

The dimeric nature of the flavans (1-3) was determined by various chemical transformations [3-5]. When the flavans were heated with 2 N HCl, cyanidin was formed (λ_{max} 530 nm); under the action of dilute mineral acid catechins were split off, the catechin for the "lower" halves of flavans (1) and (3) being (−)-epicatechin, for which the cis position of the hydrogen at C-2, and C-3 and the 2R, 3R configuration of the asymmetric centers is known, while the catechin from the "lower" half of flavan (2) was (+)-catechin — the trans isomer with the 2R, 3S configuration of the asymmetric centers.

The steric structures of the flavans of the "upper" halves were established by the formation of definite stereoisomers of the flavanols by cleavage with thioglycolic acid. In this process, from the "upper" half of the molecule a thioether is first formed, and on subsequent reduction over Raney nickel this is converted into the corresponding flavan-3-ol.

Thus, it was found that flavan (1) is a dimer of two molecules of (−)-epicatechin, flavan (2) a dimer of two molecules of (+)-catechin, and flavan (3) a combination of (+)-catechin and (−)-epicatechin. Using Weinges's nomenclature [3] they have been designated B_2, B_3, and B_4, respectively (Fig. 1).

Since the dimers themselves are very labile, we studied their stable acetyl derivatives. The PMR spectra of the decaacetates of the dimers under investigation showed the signals of the protons of eight aromatic and two aliphatic acetyl groups; i.e., not one of the ten hydroxy groups of the two catechin molecules participates in the formation of the interflavan bond (Figs. 2-4).

The spectra each contains the signals of all six protons of rings B and E, and also the three protons of rings A and D. The two protons of ring A resonate in the form of doublets with a spin–spin coupling constant (SSCC) $J_{6,8} \sim 2$ Hz, which is due to the meta interaction of these protons, and the C_6 proton of ring D in the form of a singlet, showing the absence of a second proton in the phloroglucinol ring and confirming the participation of the C_8 position of the flavan unit in the formation of the intermolecular bond in the dimer.

Only two protons of methylene groups, in place of the four present in two catechin molecules, resonate, at δ 2.88 ppm (B_2) and 2.81 (B_3 and B_4). Consequently the interflavan bond is in the C_4—C_8 positions.

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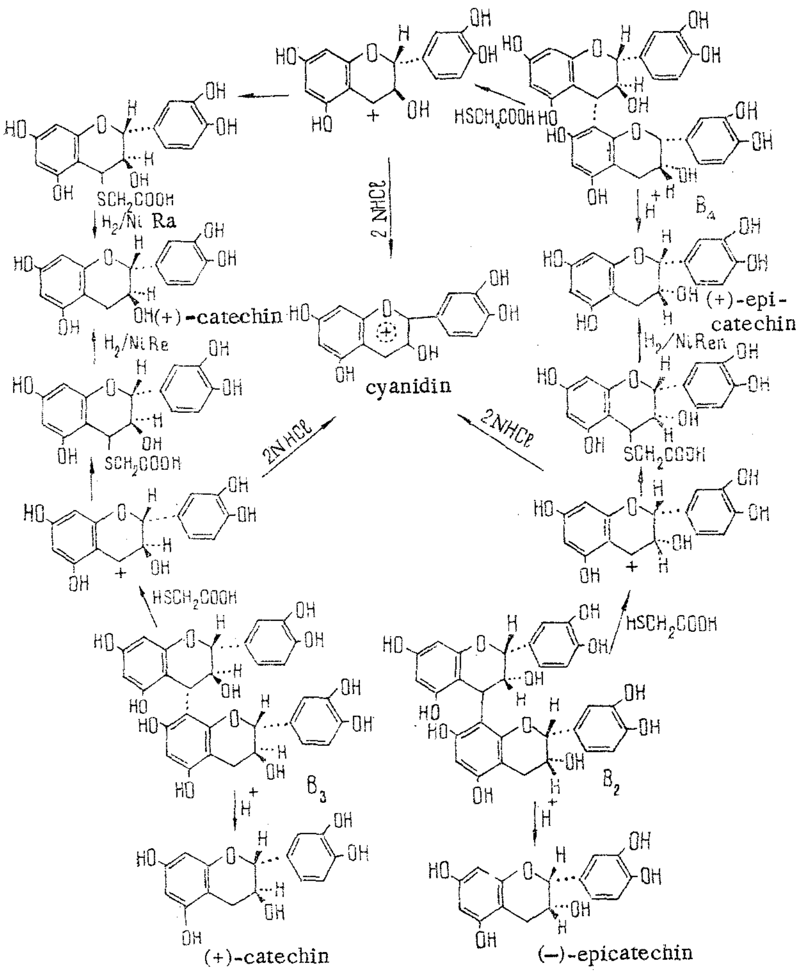

Fig. 1. Chemical transformations of the dimeric procyanidins B_2, B_3, and B_4.

The dimeric nature and C_4—C_8 form of the interflavan bond were confirmed by a mass-spectral analysis of the diacetyloctamethyl derivatives of the flavans. The mass spectra contain all the characteristic fragments confirming the proposed structures (m/z 714, 654, 492, 327, and 167), and correspond to literature information for dimeric procyanidins [3-5].

In an analysis of the PMR spectra of the decaacetates, it was found that a heterocyclic ring in the dimer molecule is present in the half-chair form with the equatorial position of the lateral phenyl ring (B) and an axial hydrogen atom at C_2.

The signal of the C_2—H proton of the "upper" half of dimer B_4 and those of the corresponding protons in both forms of dimer B_3 were recorded in the form of a doublet with an SSCC $J_{2,3} = 10$ Hz. This shows that there is a dihedral angle of ∿180° between the C_2—H and C_3—H protons and they have the trans-diaxial arrangement. The signal of the C_2—H proton of the "lower" half of dimer B_4 and those of the corresponding protons in both halves of dimer B_2 have the form of a singlet or doublet with $J_{2,3} \sim 1$ Hz. Such a weak interaction interaction of the protons shows their cis-axial-equatorial position. And the fact that the C_3—H protons of the "upper" halves of dimers B_3 and B_4 are shown in the form of a quartet with SSCCs of $J_{2,3} = 10$ Hz, and $J_{3,4} = 9$ Hz while in the dimer B_2 they form a broadened signal confirms the conclusions drawn.

In the dimers B_3 and B_4, where in the "upper" half the proton at C_3 is axial, the PMR spectra make it possible to determine the configuration and conformation of the substituents at C_4. A proton in this position gives a signal at δ 4.5 ppm in the form of a doublet with $J_{3,4} = 9$ Hz and, therefore, it is present in the transoid position with respect to the axial proton at C_3 and the absolute configuration at C_4 is S, with the "lower" flavan unit occupying the equatorial position.

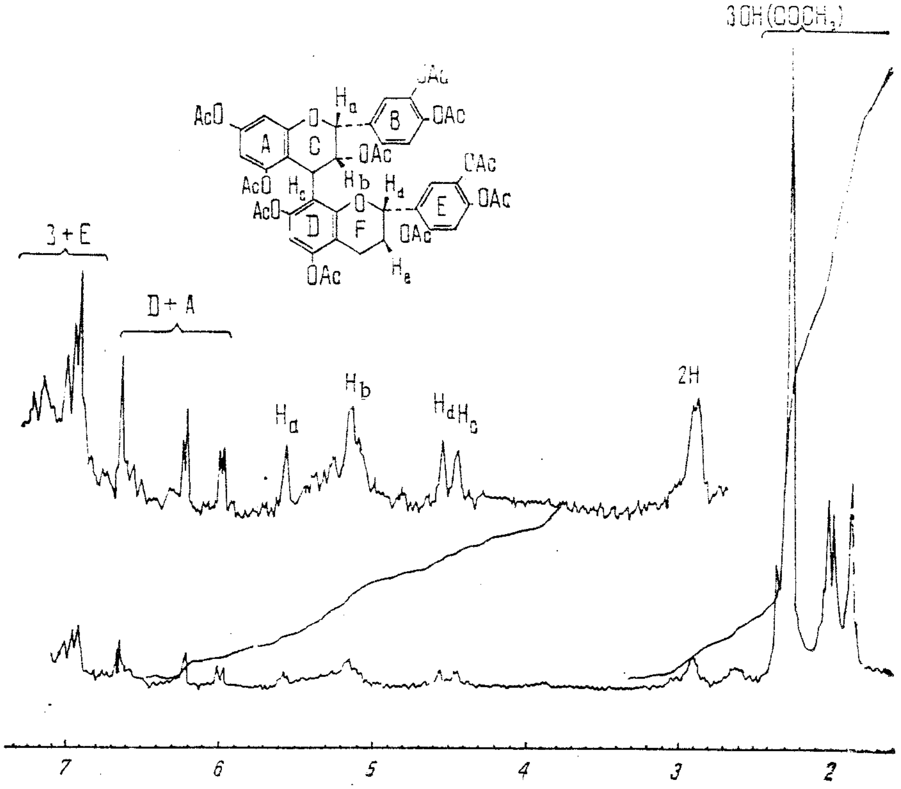

Fig. 2. PMR spectrum of the decaacetate of procyanidin B-2 ($CDCl_3$).

In dimer B_2 with the equatorial position of the proton at C_3 in the "upper" half of the molecule, the PMR spectrum does not provide the possibility of unambiguously answering the question of the configuration of the C_4 asymmetric center, since a weak interaction of the C_3—H and C_4—H protons ($J_{3,4} \sim 2$ Hz) is possible with both the axial and equatorial positions of the latter. Haslam et al. [6] has shown with the aid of ^{13}C NMR, using the γ-effect, that in dimers with an axial position of the hydroxyl at C_3, the "lower" half is also axial.

Thus, for the dimers investigated the absolute configurations of the asymmetric centers of both halves of the molecules have been determined: 2R, 3S, 4S for the "upper" half and 2R, 3S for the "lower" half of B_3; 2R, 3S, 4S for the "upper" half and 2R, 3R for the "lower" half of B_4; and 2R, 3R, 4R for the "upper" half and 2R, 3R for the "lower" half of B_2.

EXPERIMENTAL

PMR spectra were taken on a Varian HA-100D spectrometer in deuterated chloroform, and mass spectra on a MKh-1304 instrument. Specific rotations were determined on a SM circular polarimeter, and melting points on a Kofler block. Qualitative chromatographic analysis was performed on FN-3 paper in the following solvent systems: 1) butan-1-ol—acetic acid—water (40:12.5:29); 2) 2% acetic acid; 3) acetic acid—hydrochloric acid—water (5:1:1); 4) benzene—acetic acid—water (6:7:3); 5) sodium formate—formic acid—water (10:1:200); and TLC on Silufol UV-254 plates (Czechoslovakia) in system 6) benzene—acetone (8:2).

Isolation of the Dimeric Flavanols. The stems of the savin juniper, previously deresinified and defatted, were steeped with methanol for 2-3 days. The methanol was eliminated under mild conditions, and the concentrate was diluted with water, and in succession the monomeric flavans were extracted with ether and the dimeric flavans with ethyl acetate. The qualitative compositions of the fractions were determined by the PC method in systems 1 and 2.

The extractive substances from the ethyl acetate fraction were separated by absorption chromatography on a polyamide sorbent using as eluent chloroform—methanol with a gradient increase in the concentration of the latter. The dimeric flavans B_2-B_4 under investigation were desorbed with 30 and 50% methanol in chloroform. The dimers B_2, B_3, and B_4 were isolated in the individual state by repeated chromatography of the total flavans.

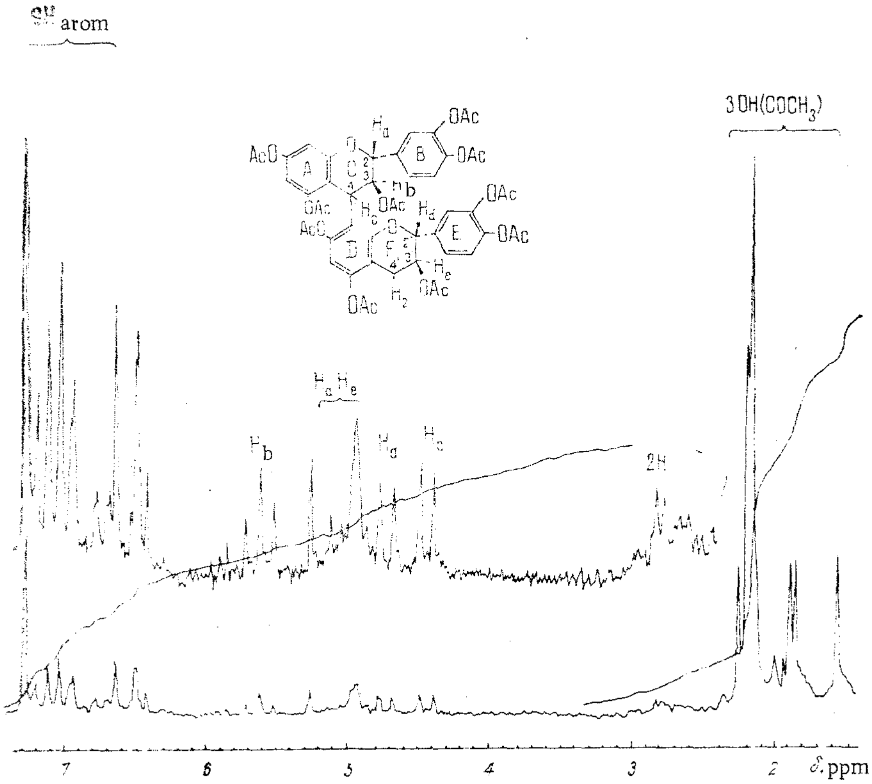

Fig. 3. PMR spectrum of the decaacetate of procyanidin B_3 ($CDCl_3$).

Anthocyanidin Test. A solution of 2 mg of the flavan under investigation in 0.5 ml of methanol was heated with 1 ml of 2 N HCl in the boiling water bath for 20 min. The reaction mixture was diluted with water, and the pigment that had formed was extracted with isoamyl alcohol and was identified from the results of UV spectroscopy (λ_{max} 530 nm) and by comparison with authentic samples of anthocyanidins by the PC method as cyanidin.

Alkaline Cleavage. A mixture of 3 mg of a flavan and 1 ml of 50% KOH was heated in the sand bath at 170°C in a current of nitrogen for 20 min. After neutralization of the reaction mixture with 25% sulfuric acid, the cleavage products were extracted with ethyl acetate. Qualitative chromatographic analysis showed that the flavans under investigation were cleaved to phloroglucinol (R_f 0.75 in system 2) and protocatechuic acid (R_f 0.10 and 0.55 in systems 4 and 5, respectively).

Acid Cleavage. A mixture of 1.5 g of a flavan and 1 ml of 0.01 N HCl was heated in the boiling water bath for 30 min. Samples were taken after 10, 20, and 30 min. The hydrolysis products were analyzed by PC in system 1 in comparison with catechin markers. After 10 min the splitting out of (—)-epicatechin from dimers B_2 and B_4 and of (+)-catechin from B_3 was observed.

Cleavage with Thioglycolic Acid. A solution of 2 mg of a procyanidin in 1 ml of ethanol was heated with 1 ml of 25% thioglycolic acid in the boiling water bath for 1 h. Samples were taken after 10, 15, 30, and 60 min, the results of the cleavage being monitored by the PC method in systems 1 and 2 in comparison with catechin labels. After 15 min, from the "bottom" halves of dimers B_2 and B_4 (—)-epicatechin and from the "bottom" half of dimer B_3 (+)-catechin had been formed. The "top" halves of the dimer molecules were converted into the thioesters of the corresponding flavan-3-ols, which were then reduced on Raney nickel catalyst (3 ml of a suspension of the catalyst in ethanol, room temperature, 2 h) to (—)-epicatechin (B_2) and (+)-catechin (B_3 and B_4).

Preparation of Acetyl Derivatives. A solution of 40 mg of a procyanidin in 3 ml of absolute pyridine was treated with 6 ml of freshly distilled acetic anhydride and the mixture was left at room temperature for 24 h. Then it was poured into ice water and the resulting mixture was kept at 4°C for 5 h. The precipitate that had deposited was separated off and

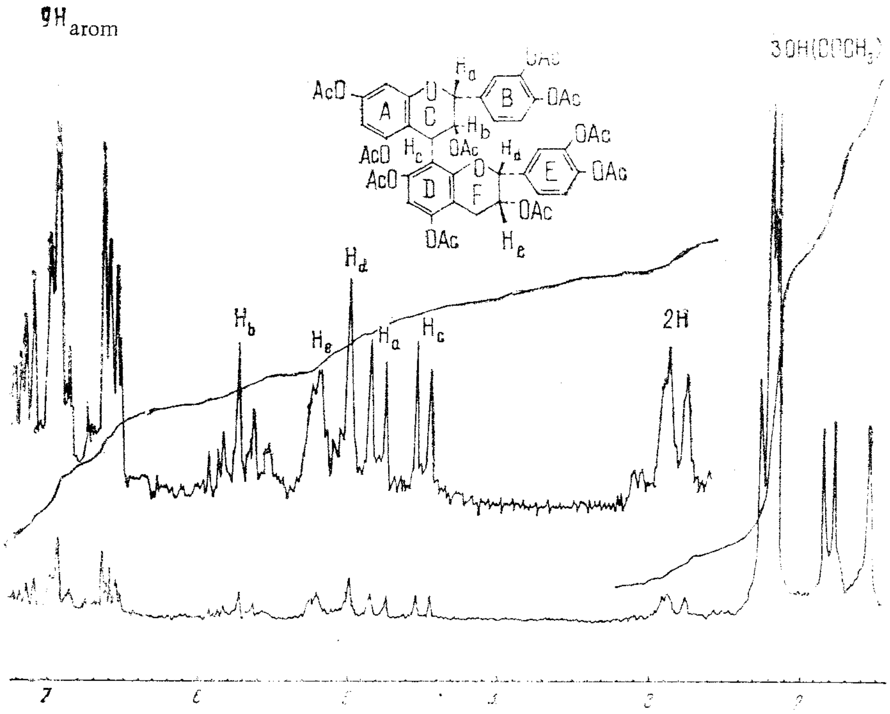

Fig. 4. PMR spectrum of the decaacetate of procyanidin B_4 ($CDCl_3$).

was purified by chromatography on a mixture of the sorbents Chromaton and silicic acid (1:5). Mixtures of benzene and chloroform in various ratios (2:1, 1:1, and 1:2) were used as eluents.

The decaacetate of procyanidin B_2 formed a white amorphous substance with the composition $C_{50}H_{46}O_{22}$, Rf 0.24 (TLC, in system 6); $[\alpha]_D^{20}$ +46.8° (c 0.6; acetone).

PMR ($CDCl_3$), δ, ppm: 2.27 and 1.88 (ArAc); 2.0 (AlAc); 6.54 and 7.04 (6 H, B and E); 5.99; 6.64 and 6.23 (3 H, A and D); 2.88 ($-CH_2-$); 5.58 (H_a); 5.15 (H_b); 4.46 (H_c); 4.56 (H_d).

The decaacetate of procyanidin B_3 formed a white amorphous substance with the composition $C_{50}H_{46}O_{22}$, Rf 0.19 (TLC, in system 6), $[\alpha]_D^{20}$ −129° (c 0.81; acetone).

PMR ($CDCl_3$), δ, ppm: 2.16 and 1.56 (ArAc); 1.85 (AlAc); 6.68-7.20 (6 H, B and E); 6.65 and 6.64 (3 H, A and D); 2.81 ($-CH_2-$); 4.65 (H_a); 4.96 (H_d, H_e); 4.45 (H_c), 5.64 (H_b).

The decaacetate of procyanidin B_4 formed colorless crystals with the composition $C_{50}H_{46}O_{22}$, Rf 0.15 (TLC, in system 6), mp 170-171°C, $[\alpha]_D^{20}$ −132° (c 0.56; acetone).

PMR ($CDCl_3$), δ, ppm: 2.18 and 1.55 (ArAc); 1.80 (AlAc); 6.90-7.24 (6 H, B and E); 6.63, 6.58, 6.52 (3 H, A and D); 2.81 ($-CH_2-$); 4.80 (H_a); 5.70 (H_b); 4.50 (H_c); 5.0 (H_d); 5.2 (H_e).

SUMMARY

Three dimeric procyanidins have been isolated from the savin juniper for the first time. On the basis of chemical transformation and the results of physicochemical and spectral analysis it has been shown that these dimers are diastereomers with different configurations of the asymmetric centers.

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COMPOSITION OF THE ESSENTIAL OIL OF *Larix sibirica*

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The composition of the essential oil isolated under production conditions from the woody verdure of *Larix sibirica* Lbd. growing in the Krasnoyarsk district has been studied. The bulk of the essential oil is composed of monoterpenes (84.95%). Among the sesquiterpene hydrocarbons α-murolene, β-humulene, and caryophyllene are present in considerable amounts. The sesquiterpene alcohol δ-cadinol has been detected. α-Murolene, γ-murolene, and longifolene have been isolated from the essential oil in the chromatographically pure state by column and preparative thin-layer chromatography.

The essential oil of *Larix sibirica* Ldb has been studied for the first time by the GLC method [1]. The raw material used was Siberian larch litter collected in July in the Tuva ASSR. The main components of the monoterpenes proved to be Δ^3-carene and α-pinene (42 and 40%, respectively).

The oil from the needles of the Siberian larch growing in Pribaikal' contained 50.4% of Δ^3-carene [2].

We have studied the essential oil of young shoots (winter) [3] and of the litter (July) [4] of a different species of larch taken in the experimental forest farm of the Voronezh Institute of Wood Technology. A paper by Kolesnikova et al. [4] is the only one which gives the composition of the sesquiterpenes of the needle oil from the Siberian larch.

All the information available in the literature related to the essential oils from the needles or from the litter of *Larix sibirica* isolated under laboratory conditions. For practical use, great interest is undoubtedly presented by the oil from the woody verdure obtained under industrial conditions.

We give the results of an investigation of the essential oil isolated by steam distillation in an industrial apparatus of the western Siberian type from the woody verdure of *Larix sibirica* growing in the Uibatskii lespromkhoz [timber industry farm], Krasnoyarsk district.

The essential oil was separated into groups of substances similar in chemical nature (acids, phenols, monoterpenes, sesquiterpenes, oxygen-containing terpene derivatives), and these were investigated further with the aid of various chromatographic and spectrometric methods.

For the general characterization of the essential oil we determined some generally adopted indices: density 0.8793 g/cm^2; refractive index, n_D^{20} 1.4730; acid No. 2.01 mg/g; saponification No. 53.27 mg/g; ester No. 51.26 mg/g.

The low values of the density and of the refractive index of the essential oil of the Siberian larch as compared with other oils indicate that the bulk of it is represented by monoterpene hydrocarbons.

The composition of the essential oil of *Larix sibirica* is given in Table 1.

The monoterpene hydrocarbons made up 84.95% of the essential oil. The predominating component — Δ^3-carene — amounted to 53.11%. The main components of the sesquiterpene hydrocarbons were α-murolene, β-humulene, and carophyllene. The amount of bornyl acetate in the

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