DIMERIC PROANTHOCYANIDINS OF *Cotoneaster oligantha*

L. T. Pashinina, T. K. Chumbalov,
V. I. Sheichenko, and R. Zh. Shukenova

UDC 547.972+978

We have investigated the dimeric proanthocyanidins of the stems of a perennial shrub of a previously unstudied species *Cotoneaster oligantha* A. Pojark, family Rosaceae.

Qualitative analysis of the polyphenols performed by the method of two-dimensional PC in solvent systems 1 and 2 and by qualitative reactions with a 1% solution of vanillin in concentrated HCl and 1% solution of ferric ammonium alum (FAA) showed the presence of six polyphenols which were provisionally assigned to the flavans. Below we give the qualitative reactions and R_f values of the flavans from cotoneaster stems:

Number of the spot	Rf in system 1	Rf in system 2	1% vanillin in conc. HCl	1% FAA
1	0,65	0,41	Orange	Green
2	0,56	0,38		
3	0,45	0,58	Pink	
4	0,43	0,50		
5	0,48	0,30		
6	0,36	0,49		

Flavans 3-5, on being heated with 2 N HCl, formed the anthocyanidin pigment cyanidin, and under the action of dilute mineral acids they formed cyanidin and catechins — in the case of flavans 3 and 5,(−)-epicatechin, and in the case of flavan 4,(−)-epicatechin and traces of (+)-catechin [1]. Thus, flavans (3-5) are dimeric proanthocyanidins in which the "lower" half of the dimer has the degree of hydroxylation and the cis configuration of the 2R:3R asymmetric centers that characterize (−)-epicatechin.

To elucidate the configurations at C_2 and C_3 of the "upper" halves of the dimers, flavans 3-5 were subjected to cleavage under the action of thioglycolic acid at room temperature followed by reduction of the thioester formed on Raney nickel. After the action of thioglycolic acid for only 1 h under these conditions the formation of (−)-epichatechin and the corresponding flavan thio derivative was observed [5, 6].

The thio derivatives of flavans 3 and 5, after the separation of the catechins, consisted of oils with light cream color having R_f 0.72 in system 1 and 0.53 in system 2, while the thio derivative of flavan 4 had R_f 0.76 in system 1 and 0.56 in system 2.

On treatment with 1% vanillin solution in concentrated HCl, the thio derivatives gave a yellow coloration that rapidly disappeared, and with a 1% solution of ferric ammonium alum a blue coloration which likewise rapidly disappeared.

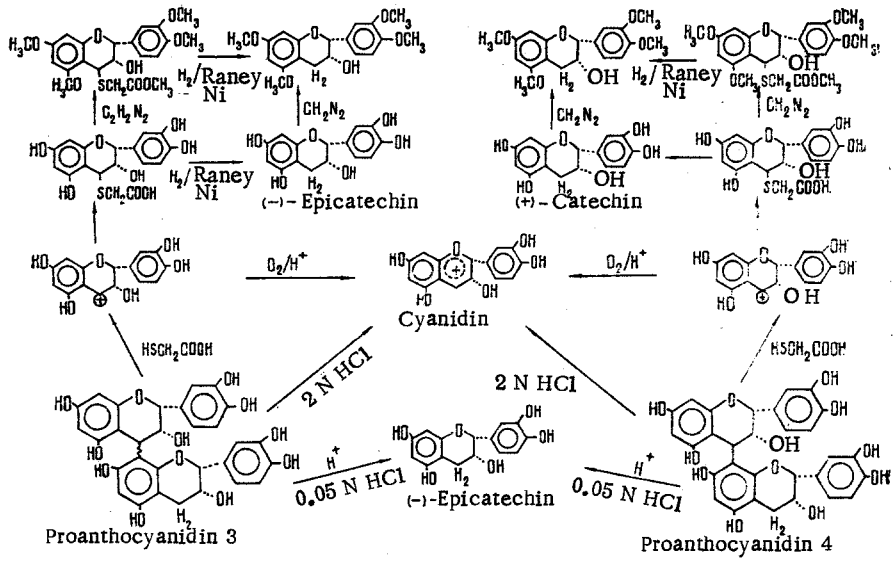
On being heated with 2 N HCl, the thio derivatives were converted into cyanidin, which shows the position of the methoxy carbonylmethylthio grouping at C_4 and the formation of this ester from the "upper" half of the molecule. On reduction over Raney nickel, the thio derivative with R_f 0.72 formed (−)-epicatechin and the thioester with R_f 0.76 formed (+)-catechin.

Methylation of the thio derivative with diazomethane followed by reduction over Raney nickel led to the formation of tetramethyl-(−)-epicatechin with R_f 0.66 in system 4 (TLC) from flavan 3 and of tetramethyl-(+)-catechin with R_f 0.55 from flavan 4, and the methyl ester of thioglycolic acid with R_f 0.89 in the same system.

The capacity for undergoing cleavage by the action of thioglycolic acid under mild conditions with the formation of a catechin and a thioester shows that this flaven is dimeric with a labile C_4-C_8 (C_6 bond) between the flavan units (see scheme on following page).

S. M. Kirov Kazakh State University, Alma-Ata. Translated from Khimiya Prirodnykh Soedinenii, No. 2, pp. 204-212, March-April, 1978. Original article submitted November 11, 1977.

 0009-3130/78/1402-0166$07.50



Chemical study of the dimeric proanthocyanidins 3 and 4

Thus, flavans 3 and 5 are dimeric proanthocyanidins in the molecule of each of which the two halves have the degree of hydroxylation and the cis configuration of the 2R:3R and 2'R:3'R centers that characterize (−)-epicatechin, and flavan 4 is also a dimeric procyanidin but its molecule has the trans configuration of the 2R:3S:4S centers of the "upper" half and the cis configuration of the 2'R:3'R centers of the "lower" half of the molecule.

Flavans 3 and 5 have the same degree of hydroxylation and the same configuration of the asymmetric centers, but differ in their chromatographic behavior and, obviously, these proanthocyanidins are stereoisomers with respect to the C_4 asymmetric center.

The acylation of flavans 3 and 4 with acetic anhydride in absolute pryidine led to the formation of decaacetates (I) and (II) with the composition $C_{50}H_{46}O_{22}$.

Methylation of the flavans with dimethyl sulfate in 50% KOH solution gave the octamethyl ethers (III) and (IV) with the composition $C_{38}H_{42}O_{12}$. On acylation with acetic anhydride in pyridine, the octamethyl ethers formed the diacetyloctamethyl derivatives (V) and (VI) with the composition $C_{42}H_{46}O_{14}$.

The NMR spectra of the decaacetates (I) and (II) taken in deuterated chloroform showed a singlet with an integral intensity of 24 protons of aromatic acetyl groups at δ 2.26 ppm for substance (I) and δ 2.28 ppm for substance (II), and also the signals of two aliphatic acetyl groups at δ 1.94 and 2.0 ppm in (I) and 1.66 and 1.90 ppm in (II). Consequently, all ten OH groups of each of the two catechin molecules are acylated and do not participate in the formation of the interflavan bond.

The spectra of substance (I) and (II) contain the signals of all six protons of rings B and E at δ 7.0-7.3 ppm (I) and at δ 6.90-7.15 ppm (II). The signals of the protons of rings A and D give two one-proton doublets at δ 6.0 and 6.4 ppm with J = 2 Hz in substance (I) and at δ 6.44 and 6.56 ppm with the same SSCC in substance (II), which characterizes a meta interaction of the protons. One-proton singlets at δ 6.65 (I) and δ 6.59 ppm (II) show the absence of the proton of a phloroglucinol ring, and, therefore, the participation of the C_8 or C_6 position of the flavonoid unit in the interflavan bond of the dimer. At δ 2.9 ppm in the spectrum of substance (I), 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 a bond (Fig. 1).

Thus, the absence of a phloroglucinol proton and of a methylene group shows the C_4-C_8 (or C_6) position of the bond.

The two C_2 protons give signals in the form of singlets (4.60 ppm, C_2-H of ring F and 5.57 ppm, C_2-H of ring C). The absence of splitting of the signals of these protons shows the cis position of the C_2 and C_3 protons on both halves of the dimer molecule. This is

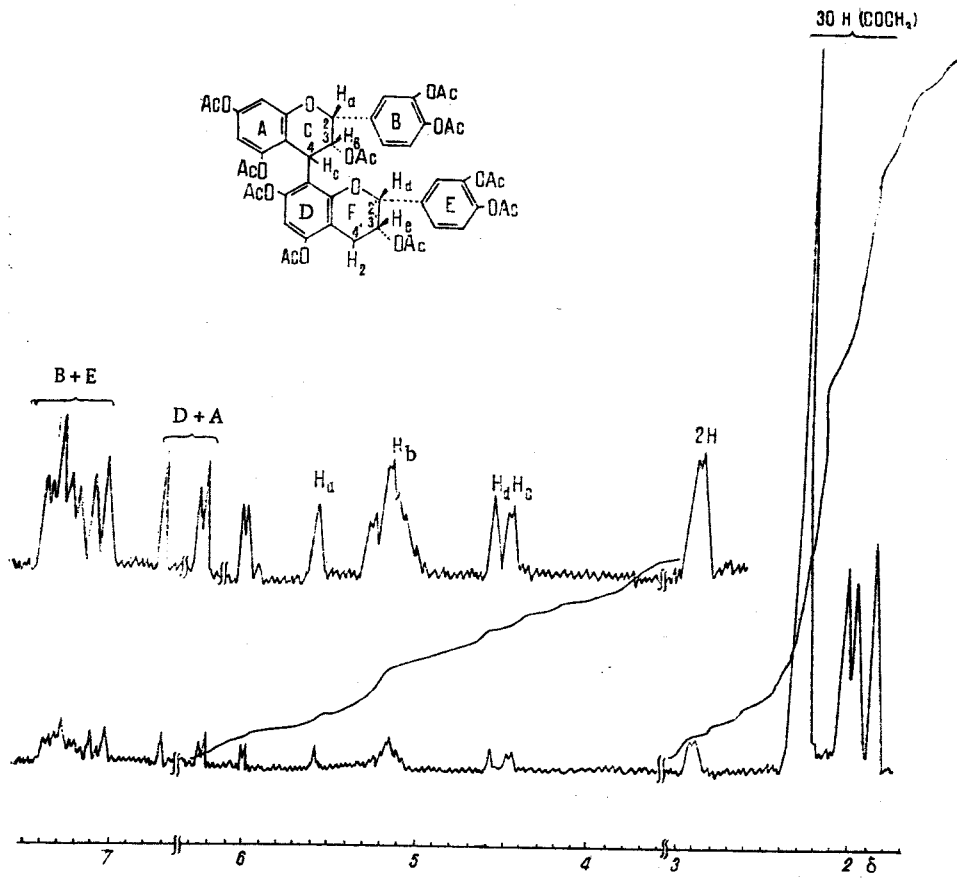


Fig. 1. NMR spectrum of the decaacetate of proanthocyanidin 3 ($CDCl_3$).

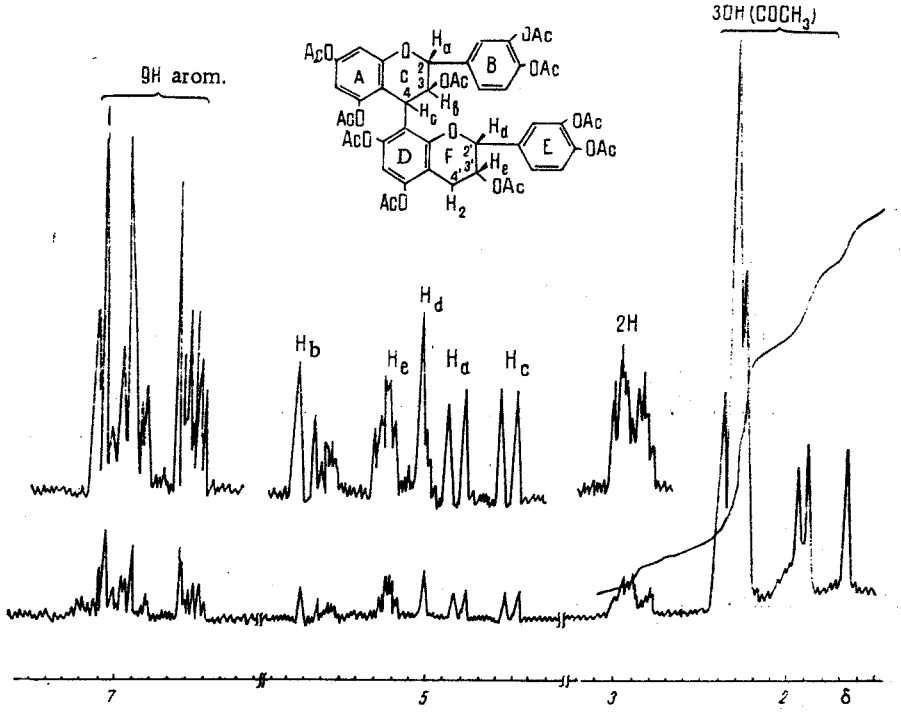


Fig. 2. NMR spectrum of the decaacetate of proanthocyanidin 4 ($CDCl_3$).

confirmed by the small SSCC of the methylene group in the "lower" half of the molecule. The C_3 protons of both heterocyclic rings resonate in the form of multiplets at δ 5.04-5.40 ppm.

The C_4 proton of the "upper" half of the molecule appears in the form of a doublet with $J_{3,4}$ = 1 Hz (4.55 ppm). Such a weak interation with the neighboring C_3 proton is possible only if the latter has the equatorial position. However, in the presence case this constant does not provide the possibility of an unambiguous answer to the question of the configuration of the C_4 proton, since both in the axial and in the equatorial position of the proton, interaction with the equatorial C_3 — H would lead to a small SSCC.

The NMR spectrum confirmed the results of chemical investigations concerning the cis position at C_2 and C_3 of both halves of dimer 3 and their 2R:3R configuration.

The C_4 proton of the "upper" half of substance (I) gives a doublet with $J_{3,4}$ = 9 Hz (4.50 ppm), corresponding to interaction with an axial C_3 proton.

The C_2 proton of the same ring appears in the form of a doublet at δ 4.8 ppm with $J_{2,3}$ = 9 Hz, and the C_3—H proton appears in the form of a quartet at 5.43-5.70 ppm. It follows from this that all three protons of ring C are trans-axial.

The protons of this ring form a singlet at 5.0 ppm (C_2—H) and a broadened singlet of C_3—H at δ 5.20 ppm, which is characteristic for cis coupling. The methylene group of substance (II) resonates in the δ 2.96 ppm region (Fig. 2).

Thus, the NMR spectra of the peracetates of the procyanidins 3 and 4 (I and II) have confirmed the results of chemical investigations that have shown that they have the structure of dimers of 3',4',5,7-tetrahydroxyflavan-3-ol with a C_4—C_8 (or C_6) bond between the flavan moieties and the cis configuration of the C_2 and C_3 asymmetric centers of both halves of the molecule, i. e., the 2R:3R configuration in the case of procyanidin 3 and the trans configuration of the asymmetric centers (2R:3S:4S) of the "upper" half and the cis configuration (2'R:3'R) of the "lower" half of the molecule in the case of procyanidin 4.

To prove the dimeric structure we also used mass-spectrometric analysis, which apart from confirming the mass, gives information on the lability and position of the C—C bond between the monomeric parts of the molecule.

The mass peaks M^+ 774 in the spectra of the diacetyloctamethyl derivatives of dimers 3 and 4 correspond to the calculated molecular weight for $C_{42}H_{46}O_{14}$ [2, 4].

Retrodiene cleavage, elimination of acetic acid, and cleavage at the labile C—C bond give the main fragments in the mass spectrum. The elimination of acetic acid leads to a flavene with m/e 714 which, as the result of retrodiene cleavage, gives a fragment with m/e 492. In its turn, the fragment with m/e 492, as a 4-substituted polymethoxyflavene, in accordance with the general scheme of cleavage [7], gives fragments with m/e 327 and 165, and 355 and 137.

The double elimination of acetic acid gives a diflavene with m/e 654 which can be cleaved into two flavylium ions with the same mass, 327, and shows the cleavage of the C_4—C_8 (or C_6) bond. The mass spectra of the substances investigated are in harmony with literature information [2, 4, 7].

EXPERIMENTAL

The specific rotations were determined on a CM circular polarimeter and the melting points on a Kofler block; the NMR spectra were taken on a Varian HA-100D instrument in deuterated chloroform, the chemical shifts being given in the δ scale. The mass spectra were taken on a MKh-1304 instrument with a system for direction introduction, with heating. The energy of the ionizing electrons was 70 eV. As adsorbents we used polyamide, silicic acid, Chromaton N (0.16-0.20; (Czechoslovakia), and Silufol UV-254 prepared plates for TLC (Czechoslovakia).

The solvent systems for PC were: 1) butan-1-ol—acetic acid—water (40:12.5:29); 2) 2% acetic acid; and 3) acetic acid—hydrochloric acid—water (5:1:5); and for TLC: 4) benzene—acetone (8:2) and 5) ethyl acetate—chloroform (1:1).

Isolation of the Flavans. The air-dry woody part of the stems of the cotoneaster (5kg) was comminuted to a particle size of 0.5-1 cm and was treated successively with benzene and chloroform to eliminate resins and waxes. The flavans were extracted from the raw material by steeping with methanol for two days. The methanolic concentrate after dilution with water was extracted successively with ether and with ethyl acetate until the reaction with vanillin

was negative. The ethereal extracts were dried with calcined magnesium sulfate and evaporated to dryness. As PC in systems 1 and 2 showed, the ether extract contained flavans 1 and 2 and resins, and the ethyl acetate extract contained flavans 2-6 and traces of 1. Flavans 1 and 2 were isolated from the ethereal extract. Their separation was achieved by partition chromatography of silica gel. The substances from the ethyl acetate fraction were separated by adsorption chromatography on polyamide. Catechins 1 and 2 were eluted by 10% methanol, catechins and traces of flavans 3, 4, and 5 by 30% methanol, flavans 3, 4, and 5 by 50% methanol, and flavan 6 by 100% methanol. Rechromatography of the substances from the 50% fraction gave flavans 3-5. The sequence of elution of the flavans from the polyamide was 3→4→5.

Flavan 1, (+)-catechin, formed a colorless crystalline substance from water with mp 176-177°C; $[\alpha]_D^{21}$ +16° [c 0.98; acetone—water (1:1)]. (+)-catechin pentaacetate formed colorless needles with mp 131-132°C; $[\alpha]_D^{21}$ +33.1° (c 0.68; chloroform). Tetramethyl-(+)-catechin formed colorless needles with mp 143-144°C; $[\alpha]_D^{21}$ −13.4° (c 0.89; chloroform).

Flavan 2, (−)-epicatechin, formed colorless prisms from water with mp 231-233°C, $[\alpha]_D^{21}$ −58.9° [c 0.48; acetone—water (1:1)]. (−)-Epicatechin pentaacetate formed colorless needles from ethanol with mp 150-151°C; $[\alpha]_D^{20}$ 16.0 (c 0.98; chloroform). Tetramethyl-(−)-epicatechin formed colorless needles from ethanol with mp 151-152°C; $[\alpha]_D^{20}$ −61.5° (c 0.7; chloroform).

Conversion into an Anthocyanidin. A solution of 5 mg of a proanthocyanidin in 1.5 ml of methanol was heated with 2 N HCl in methanol on the boiling water bath for 20 min. The reaction mixture acquired a red coloration. After dilution with water, the pigment was extracted with isoamyl alcohol. Chromatography in system 3 together with anthocyanidin markers showed identity with an authentic sample of cyanidin having R_f 0.34, λ_{max} 525 nm in ethanol, for each of the substances.

Acid Degradation. A solution of 4 mg of a proanthocyanidin in 1 ml of methanol was heated with 0.05 N HCl solution in the water bath for 30 min. After 10, 20, and 30 min the reaction products were extracted with ether. The composition of the ethereal fractions was determined by PC in systems 1 and 2 with catechin markers. On treatment with 1% solution of vanillin in concentrated HCl, after 10 min (−)-epicatechin was detected in all the flavans investigated, and in the case of flavan (4) traces of (+)-catechin, as well.

Cleavage with Thioglycolic Acid. A solution of 5 mg of one of the proanthocyanidins in 1.5 ml of ethanol was treated with 1.5 ml of thioglycolic acid. The reaction was carried out at room temperature in a current of nitrogen for 24 h. Samples were taken after 1, 2, 4, 6, and 24 h. The reactions products were analyzed by one-dimensional PC with markers in system 1. As a result of cleavage, after only 1 h (−)-epicatechin was obtained from all the flavans investigated, being identified by comparison with an authentic sample, and flavan thio derivatives with R_f 0.72 in the case of flavan 3 and R_f 0.76 in system 1 in the case of flavan 4 were also obtained.

Isolation of the Methoxycarbonylmethylthio Derivatives. To isolate the thio derivatives of flavans 3,4, and 5, the reaction mixture was treated with a saturated solution of sodium bicarbonate and was extracted with ethyl acetate. The ethyl acetate fraction was found by PC to contain (−)-epicatechin. The aqueous phase was acidified and extracted with ethyl acetate. After the ethyl acetate had been distrilled off, the thio derivatives were obtained in the form of oils with R_f 0.72 in system 1 and 0.53 in system 2 (PC) in the case of flavan 3 and with R_f 0.76 and 0.56 in the case of flavan 4, these substances giving a rapidly disappearing blue coloration with a solution of ferric ammonium alum and a yellow coloration, likewise rapidly disappearing, with vanillin.

To 3 mg of a thioester was added 3 ml of a suspension of Raney nickel catalyst in ethanol, and the mixture was kept at room temperature for 2 h. The catalyst was filtered off and the reduction products were analyzed by PC with catechin markers in the systems mentioned above. (+)-Catechin was found in the reduction products of flavan 4 and (−)-epicatechin in those of flavans 3 and 5.

Conversion into Anthocyanidins. A procyanidin thioester (2 mg) was heated with 2.5 ml of 3 N HCl in methanol solution on the water bath for 20 min. The formation of a red coloration took place which passed into a layer of isoamyl alcohol on extraction. Comparison with authentic samples of anthocyanidins on chromatography in system 3 showed the presence of cyanidin.

The methylation of the thio derivatives was performed with an ethereal solution of diazomethane twice. After the elimination of the ether, an oil was obtained which was chromatographically homogeneous with R_f 0.80 in system 5 (TLC). Then this substance was reduced at room temperature on Raney nickel for 2 h whereupon, as TLC showed, substances with R_f 0.66 and 0.90 were formed from flavan 3 and with R_f 0.55 and 0.90 from flavan 4. To separate these substances they were chromatographed on a column of Chromaton—silicic acid (1:5) with elution by benzene. After two separations the following substances were obtained: with R_f 0.66, which was identical with the tetramethyl-(−)-epicatechin marker, with R_f 0.56, identical with the tetramethyl-(+)-catechin marker, and with R_f 0.90, corresponding to methyl thioglycolate.

Acetylation of the Proanthocyanidins. A proanthocyanidin (150 mg), dried over P_2O_5, was dissolved in 3 ml of absolute pyridine and acylated with 5 ml of freshly distilled acetic anhydride at room temperature for 24 h. The solution was poured into cooled water and allowed to stand at 4°C for 5 h. The precipitate that had deposited was separated off, washed with water, and purified on a column of Chromaton—silicic acid (1:5) using as eluents mixtures of benzene and chloroform (1:1 and 1:2).

The decaacetate of procyanidin 3 formed a white substance with the composition $C_{50}H_{46}O_{22}$, mp 128-130°C; $[\alpha]_D^{20}$ +48.7° (c 1.08; acetone); R_f 0.32 in system 4 (TLC).

NMR ($CDCl_3$), δ, ppm: 2.26 (ArAc), 1.94 and 2.0 (AlfAc) 7.0-7.3 (3H, B, and E), 6.0 and 6.4; 6.65 (3H A and D), 2.9 ($-CH_2-$), 5.0-5.4 (Hb); 5.57 (Ha); 4.60 (Hd); 4.55 (Hc).

The decaacetate of procyanidin 4 formed colorless prisms collected into druses, from ethanol, with the composition $C_{50}H_{46}O_{22}$; mp 170-172°C; $[\alpha]_D^{21}$ −113° (c 1.08; acetone); R_f 0.18 in system 4 (TLC).

NMR ($CDCl_3$), δ, ppm: 2.28 (ArAc), 1.66 and 1.90 (AlfAc), 6.90-7.15 (3H, B and E); 6.44; 6.56; 6.59 (3H, A and D), 2.96 ($-CH_2-$); 5.0 (Hd); 5.20 (He); 4.50 (Hc); 4.80 (Ha); 5.43-5.70 (He).

Methylation. To 100 mg of the decaacetate of procyanidin 3 in 5 ml of methanol were added 4 ml of dimethyl sulfate and, carefully in drops with stirring, avoiding overheating, 6 ml of 50% KOH solution. After the end of the reaction, the mixture was poured into ice water and was allowed to stand at 0°C for 3 h. The resulting mixture was extracted with chloroform. The methyl derivative obtained after the chloroform had been distilled off was purified by chromatography on a column of Chromaton—silicic acid (1:5). Elution was performed with mixtures of benzene and chloroform in ratios of 1:1 and 1:2. Two chromatographic separations yielded the octamethyl ether of procyanidin 3 in the form of an amorphous substance with $[\alpha]_D^{20}$ +88.2° (c 0.17; acetone) with R_f 0.30 in system 4 (TLC).

Diacetyloctamethyl Derivative of Procyanidin 3. A solution of 30 mg of the octamethyl ether in 2 ml of absolute pyridine was treated with 4 ml of freshly distilled acetic anhydride and left to stand at room temperature for 24 h. The reaction mixture was poured into ice water and allowed to stand at 5°C for 5 h. The precipitate that had deposited was filtered off, washed, and purified on a column of Chromaton—silicic acid (1:5) under the conditions described above. It was crystallized from methanol, giving faintly yellowish crystals with mp 133-135°C, R_f 0.17 in system 5 (TLC).

Mass spectrum: M^+ 774 (30%), 714 (100%), 654 (0%), 553 (29%), 492 (50%), 327 (52%), 355 (40%), 222 (30%), 180 (30%), 150 (20%).

The octamethyl ether of procyanidin 4 was obtained by methylating 25 mg of the decaacetate with 3 ml of dimethyl sulfate in methanolic solution by the method described above. After purification, an amorphous substance with R_f 0.22 in system 4 was obtained.

The diacetyloctamethyl derivative of procyanidin 4 was obtained by acylating 18 mg of the octamethyl ether with 4 ml of acetic anhydride in 25 ml of absolute pyridine at room temperature in the same way as the flavan 3 was acylated. The product obtained was purified on a column of Chromaton—silicic acid (1:2). The final product was a white amorphous substance with R_f 0.15 in system 4.

Mass spectrum: M^+ 774 (67%), m/e: 714 (100%), 732 (57%), 654 (89%), 492 (56%), 355 (31%), 327 (52%), 222 (32%), 180 (29%), 150 (23%).

SUMMARY

1. Five flavans have been isolated from the stems of *Cotoneaster oligantha* A. Pojark, and have been identified; two of them are (+)-catechin and (−)-epicatechin and three are stereoisomeric dimeric procyanidins.

2. The proanthocyanidins have the structure of dimers of 3',4',5,7-tetrahydroxyflavan-3-ol with C_4-C_8 (or C_6) bonds between the flavan moieties and with the cis (2R:3R) configuration of the asymmetric centers (flavans 3 and 5) and trans (2R:3S:4S) configuration of the "upper" half and the cis configuration of the "lower" half of the molecule (flavan 4).

LITERATURE CITED

1. T. A. Geissman and H. F. K. Dittmar, Phytochemistry, 4, 359 (1965).
2. K. Weinges, W. Bähr, W. Ebert, K. Göritz, and H.-D. Marx, Fortschr. Chem. Org. Naturstoff, 27, 158 (1969).
3. K. Weinges, W. Kaltenhäuser, H.-D. Marx, E. Nader, F. Nader, J. Perner, and D. Seiler, Ann. Chem., 711, 184 (1968).
4. K. Weinges, K. Göritz, and F. Nader, Ann. Chem., 715, 164 (1968).
5. R. S. Tompson, D. Jacques, E. Haslam, and R. J. V. Tanner, J. Chem. Soc., Perkin I, 11, 1387 (1972).
6. K. D. Sears and R. L. Casebir, Chem. Commun., 1437 (1968).
7. L. T. Pashinina, N. D. Storozhenko, and T. K. Chumbalov, Khim. Prirodn. Soedin., 735 (1976).