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TANNINS FROM LEAVES OF Hippophaë rhamnoides

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Four individual hydrolyzable tannins have been isolated for the first time from the leaves of the common sea buckthorn, two of which have been identified as strictinin and isostrictinin. The structure of the new tannin hipporhamnin has been established (6-O-galloyl-1,3-O-hexahydroxydiphenoyl-β-D-glucose). It has been shown that the free gallic acid in the leaves of the common sea buckthorn is an artifact.

The chemical composition of the leaves of the common sea buckthorn is complex. In them have been found flavonoids [1-4], carotenoids [6], amino acids [6], triterpene compounds [7, 8], sterols [7], coumarins [3], alkaloids [9], quebrachitol [11], gallic acid [3], and tannins [3, 10]. The amount of tannin substance in sea buckthorn leaves exceeds 10% [10, 12, 13].

From the tannin fraction of the leaves of Hippophaë rhammoides (common sea buckthorn) we have isolated four individual substances belonging to the group of hydrolyzable gallo-ellagotannins which give a positive reaction with ferric chloride and with sodium nitrite for bound ellagic (hexahydroxydiphenic) acid [15, 16]. Under the action of a dilute mineral acid, these substances hydrolyze with the formation of gallic and ellagic acids and glucose. In other compounds, the ratio of the components mentioned was 1:1:1. This ratio was confirmed in each case by the ^{1}H NMR spectrum. Tannins isolated from various plants with the same ratio of gallic and hexahydroxydiphenyl groups and glucose are known [17-27]. Compound (I) was close in the value of its specific optical rotation and the rate of hydrolysis to juglanin, the structure of which has not been determined [26].

In the ^{1}H NMR spectrum of (I) (deuteroacetone, TMS), the protons of the gallic acid residue gave a signal at 7.12 ppm (s, 2 H) and two protons of the hexahydroxydiphenyl residue gave signals at 6.50 and 6.78 ppm (singlets, 1 H). The four signals of the protons of the glycoside moiety of (I), the assignment of which was made with the aid of double resonance, were present at 5.65 ppm, $J_{1,2}$ = 4.8 Hz (H_1); 4.70 ppm, $J_{2,3}$ = 2 Hz (H_2); 4.08 ppm, $J_{6,5}$ = 2.9 Hz; $J_{6,6'}$ = 13.3 Hz; and 4.82 ppm, $J_{6',5}$ < 1 Hz (H_6 and $H_{6'}$). The signals of the remaining three protons (H_3, H_4, and H_5) were observed in a narrow region of the spectrum (5.2-5.4 ppm). The signals of two protons (H_4 and H_5) could be approximated by an AB quartet ($J_{4,5}$ = 9.7 Hz), the components of which were separated as the result of the interaction of the H_4 proton (5.43 ppm, $J_{4,3} \approx 2.0$ Hz) with the H_3 proton (5.40 ppm) and of the H_5 proton (5.30 ppm) with the proton of a methylene group giving a signal in the weak field. The assignment of the signal at 5.43 ppm (H_4), was made on the basis of the results of an analysis of the intensities of the lines of this signal ($\Delta\nu_{3,4} \approx J_{3,4}$). Below, we give the chemical shifts δ_i (ppm) and coupling constants $J_{i,i+1}$ (Hz) of the protons in the glucopyranose moiety of compound (I):

H	*1*	*2*	*3*	*4*	*5*	*6*	*6′*
δ_i	5.65	4.70	5.40	5.43	5.30	4.08	4.82
$\delta_{i,i+1}$	1.2	2 3	3,4	4,5	5,6	6.6′	6′,5
J, Hz	4,8	9,0	2,0	9,7	2.9	13,3	0

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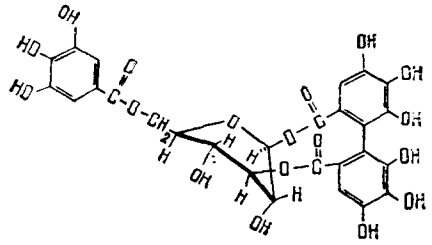
TABLE 1. Mass Numbers of the Main Peaks of the Peracetyl Derivative of Hipporhamnin

m/z	Intensity, %	m/z	Intensity, %	m/z	Intensity, %
1012	3,2	690	12,0	447	14,0
994	2,8	698	8,0	446	12,0
970	2,0	674	29,0	429	32,0
952	6,0	656	16,0	428	27,0
928	2,0	632	16,0	387	42,0
910	4,4	614	17,0	386	37,0
868	2,6	572	23,0	344	52,0
850	0,8	554	11,0	302	100,0
808	0,8	530	18,0	237	32,0
801	2,4	512	14,0	226	68,0
800	2,4	488	15,0	211	51,0
758	4,0	471	36,0		
716	20,0	470	34,0		

Thus, of the vicinal constants of the glucose protons, only $J_{4,5}$ had a large value, which, according to [19], indicates the existence of the glucose ring in the 2B conformation. The position of attachment of the hexahydroxydiphenyol residue determines the conformation of the carbohydrate ring [20]. In the 2B conformation of the glucopyranose ring of compound (I), the oxygen atoms in positions 1 and 3 are spatially close, which can only be the result of the attachment of the hexahydroxydiphenoyl group at these centers. The considerable downfield shift of the signals of the methylene group and the chemical shift between them ($\Delta\delta_{6,6'}$ = 0.8 ppm) is not the result of an influence of the hexahydroxydiphenoyl group, since this group is spatially remote from the methylene group. The values of the chemical shifts of the protons of the methylene group are extremely close to those in the molecule of a tannin with a galloyl group in position 6 [25]. These facts indicate that the galloyl group in the compound under consideration is likewise present in position 6. The descreening of the H_4 proton is, in all probability, due to the influence of the oxygen atom in position 3, in relation to which the H_4 proton is present in the eclipsed conformation. Consequently, compound (I) is 6-O-galloyl-1,3-O-hexahydroxydiphenoyl-β-D-glucose, and we have called it hipporhamnin.



The electron impact mass spectrum of the peracetyl derivative of (I) (Table 1) lacked the peak of the molecular ion, which is connected with the high molecular weight of the substance (>1000 m.u.) and is in harmony with literature information [28] for analogous compounds. The fragmentation of the $M^{+\cdot}$ ion of peracetyl-(I) takes place in several directions. One of the decomposition pathways is connected with the elimination of triacetylgallic acid in the form of a neutral molecule or a triacetylgalloyl radical with the subsequent successive splitting out of acetyl groups in the form of ketene and/or actic acid molecules [28]. Another decomposition pathway consists in the successive elimination of acetyl groups, again in the form of ketene and/or acetic acid molecules. The peak with the maximum mass in the mass spectrum of the peracetyl derivative of (I) is the peak of an ion with m/z 1012, corresponding to the loss of two ketene molecules by the molecular ion. The peaks of ions with m/z 994, 970, 952, and others connected with the further ejection of acetyl groups are shown in Table 1. The subsequent breakdown of these ions leads to the formation of the strongest radical ion – that of ellagic acid – with m/z 302, which is formed from the hexahydrodiphenic acid residue in the molecule of the acetyl derivative of (I).

Tannins (II) and (III), which have been isolated for the first time from sea buckthorn leaves were identified as strictinin [21-23] and isostrictinin [23-25], respectively, on the basis of a comparison of their empirical formulas, angles of optical rotation, and IR and ^1H NMR spectra with literature information. The study of tannin (IV) is continuing.

It has been reported that free gallic acid has been isolated from the leaves of Hippophaë rhamnoides L. [3]. However, according to the results of chromatography in a thin layer of type Silufol silica gel (Czechoslovakia) and cellulose (Filtrak) there is no free gallic acid in freshly gathered leaves of the common sea buckthorn. This substance is formed on the prolonged standing of aqueous extracts of the sea buckthorn or when they are heated or treated with acidic reagents.

EXPERIMENTAL

Common sea buckthorn leaves collected in September in Moscow Oblast were investigated.

The chromatographic purities and mobilities of the tannins and the gallic and ellagic acids were determined by TLC on Merck cellulose in system 1) butan-1-ol–acetic acid–water (20:5:9) in 10% acetic acid. The substances were detected with a 1% ethanolic solution of ferric chloride. The chromatographic purity of the acetyl and methyl derivatives of hipporhamnin was monitored by TLC on standard Silufol silica plates (Czechoslovakia) in systems 2) chloroform–methanol–water (20:0.25:0.02), with a revelation by a 10% solution of potassium or sodium hydroxide in ethanol, and 3) chloroform–methanol–water (18:0.15:0.02), with revelation in UV light at 360 nm. The chromatograhic mobility of glucose was determined on a plate coated with KSK silica gel (the silica gel was deposited from a 0.3 M solution of NaH_2PO_4, and the plates were kept at 105°C for 30 min) in system 4) butan-1-ol–methanol–water (5:3:1), with detection by means of an ethanolic solution o-toluidine acetylsalicylate, followed by heating the plate at 105°C for 10 min, and on F-16 paper (GDR) in system 5) butan-1-ol–pyridine–water (6:4:3), with detection by aniline hydrogen phthalate.

^1H NMR spectra were obtained on Varian XL-200 and Bruker WM-360 NMR spectrometers (deuteroacetone, $CDCl_3$, 0 – TMS); IR spectra on a Specord 75 IR; UV spectra on a Spectra M 40; and mass spectra on Varian MAT 44 mass spectrometer with direct introduction of the sample into the ion source at the emitter. The temperature of the ionization chamber was 175°C, the ionizing energy 70 eV, and the emission current 0.8 mA. The emitter was heated from 5 to 300°C at the rate of 220°C/min. Melting points were determined on a Boëtius stage.

Isolation of the Tannins. The tannins were isolated by the usual method from the fraction of polar substances, using the chromatographic separation of the components. From 200 g of dry sea buckthorn leaves was obtained a total of 3.14 g of extractive substances, from which four individual tannins were isolated, with R_f 0.12, 0.19, 0.32, and 0.4. The yield of the tannin with R_f 0.12 (I) was 2.5%, that with R_f 0.32 (II), 0.21%, and that with R_f 0.4 (III), 0.19% (calculated on the air-dry raw material). The tannin with R_f 0.19 was present in minor amounts.

Tannin (I) (hipporhamnin) with the composition $C_{27}H_{22}O_{18}$ was an amorphous sandy-colored powder, $[\alpha]_D$ +41° (c 0.40; methanol). IR spectrum (ν, cm^{-1}, paraffin oil): 1620, 1720 (C=O), 3100-3600 (OH). Found, %: C 49.76; H 3.98; Calculated, %: C 49.8; H 3.70; M 652, $C_{27}H_{22}O_{18}\cdot H_2O$. Compound (I) gave a positive reaction for bound ellagic acid [15, 16].

Acid Hydrolysis of Hipporhamnin. A solution of 0.6 g of hipporhamnin (I) in 0.1 N sulfuric acid (5.0 ml) was heated under reflux in the boiling water bath for 11 h. On cooling, the reaction mixture deposited a crystalline product A (0.276 g). Substance A was crystallized from a mixture of aqueous pyridine and ethanol, mp 360°C, M 302 (mass-spectrometrically), and it gave a positive reaction for free ellagic acid [14]. The R_f values of compound A and an authentic sample of ellagic acid coincided. The acid aqueous filtrate was extracted repeatedly with ether. This gave a crystalline substance B (0.145 g), which was purified by chromatography on silica gel 40/100 (elution by ether), the melting point of the compound isolated being 247-248°C. It was identified with an authentic sample of gallic acid. On methylation with diazomethane, the methyl ester of trimethylgallic acid was obtained, with mp 69-70°C, M 226 (mass-spectrometrically). The aqueous mother solution was evaporated to dryness (C). The dry residue was chromatographed on plates coated with KSK silica gel in system 4 and on FN-16 paper in system 5 with an authentic sample of glucose, and the R_f values of the authentic sample of glucose and of C coincided.

Methylation of Hipporhamnin (I). An excess of an ethereal solution of diazomethane was added to 0.20 g of (I) in 2 ml of methanol, and the reaction mixture was left for 72 h. According to TLC, the reaction product (0.232 g) consisted of four substances. A chromato-

graphic check was made on Silufol plates in system 3. The mixture of substances obtained (0.232 g) in 3 ml of methanol was treated with 0.02 g of Ag_2O and 2 ml of CH_3I, and the mixture was boiled under reflux for 3.5 h. The dry residue after the solvent had been evaporated off was chromatographed on a column of Woelm silica gel in chloroform. The chloroform fraction (0.11 g) was chromatographed on a column of the same sorbent with elution by diethyl ether–heptane (1:9). This gave undecamethylhipporhamnin with mp 78-79°C (heptane) (0.08 g), the IR spectrum of which lacked the absorption band of hydroxy groups. Found, %: C 57.78; H 5.7; M 788. $C_{38}H_{44}O_{18}$. Calculated %: C 57.86; H 5.58; M 788. $C_{38}H_{44}O_{18}$.

Acetylation of Hipporhaminin (I). A mixture of 0.06 g of (I), 3 ml of acetic anhydride, 2 ml of pyridine, and 0.25 g of sodium acetate was heated under reflux in a boiling water bath for 28 h. The reaction mixture was poured into cold water, and the precipitate that deposited was filtered off and was chromatographed on a column of Woelm silica gel in chloroform. The dry residue after the elimination of the solvent was crystallized from ethanol. This gave undecaacetylhipporhamnin with mp 177-178°C. The individuality of the substance was checked by chromatography on Silufol in system 2. Its IR spectrum lacked the absorption band of hydroxy groups. ^1H NMR spectrum (200 MHz, $CDCl_3$, TMS, δ scale): 1.98-2.32 (11 $OCOCH_3$); 5.86 (1 H, H_1); 4.16 (1 H, H_4); 3.93 (1 H, H_6); 7.80 (s, 2 H of a galloyl residue); 7.33 and 7.46 (s, s, 1 H, 1 H, hexahydroxydiphenoyl residue).

Tannin (II) with the composition $C_{27}H_{22}O_{18}$, formed an amorphous sandy-colored powder, $[\alpha]_D$ −6° (c 0.5; methanol); IR spectrum (ν, cm^{-1}, paraffin oil): 3000-3600, 1710, 1600, 1510, 1460, 1380, 1300, 1170, 1095, 1045, 1015. ^1H NMR spectrum (360 MHz, Me_2CO-d_6, TMS, δ, ppm): 7.15 (2H, s, galloyl residue); 6.57 and 6.70 (1H, s, hexahydroxydiphenoyl residue); 5.69 (1H, d, H-1), $J_{1,2}$ = 7.9 Hz; 3.63 (1H, t, H-2); 3.73 (1H, t, H-3), $J_{2,3}$ = 8 Hz; 4.87 (1H, t, H-4), $J_{3,4}$ = 9.7 Hz; 4.08 (1H, q, H-5), $J_{4,5}$ = 9.7 Hz; 3.85 (1H, d, H-6); 5.28 (1H, q, H-6'), $J_{5,6}$ = 6.5 Hz, $J_{6,6'}$ = 13.5 Hz; it was identified as strictinin.

Tannin (III), with the composition $C_{27}H_{22}O_{18}$, formed an amorphous sandy-colored powder, $[\alpha]_D$ −8° (c 0.4; methanol). IR spectrum (ν, cm^{-1} paraffin oil): 3000-3600, 1720, 1610, 1445, 1515, 1360, 1310, 1290, 1065-1040. ^1H NMR spectrum (360 MHz, Me_2CO-d_6, TMS, δ, ppm): 7.15 (2H, s, galloyl residue); 6.42 and 6.71 (1H, hexahydroxydiphenoyl residue); 6.13 (1H, d, H-1); 5.00 (1H, t, H-2), $J_{1,2}$ = 8.5 Hz; 5.21 (1H, t, H-3), $J_{2,3}$ = 9.4 Hz; 3.95 (1H, t, H-4), $J_{3,4}$ = 9.5 Hz; 3.7-4.0 (t, H-5, H-6, and H-6'); it was identified as isostrictinin.

SUMMARY

Four individual hydrolyzable tannins have been isolated for the first time from common sea buckthorn leaves, and two of them have been identified as strictinin (1-O-galloyl-4,6-O-hexahydroxydiphenoyl-β-D-glucose) and isostrictinin (1-O-galloyl-2,3-O-hexahydroxydiphenoyl-β-D-glucose, respectively.

The structure of a new tannin – hipporhamnin – has been established as 6-O-galloyl-1,3-O-hexahydroxydiphenoyl-β-D-glucose.

It has been shown that the free gallic acid in common sea buckthorn leaves is an artifact.

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