LISTVENOL – A NEW FLAVONOID FROM THE BARK OF Larix sibirica

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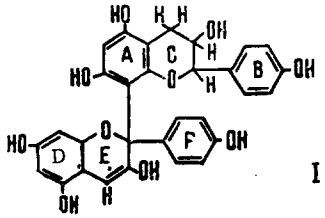
UDC 547.972

We have reported previously the detection in the bark of Larix sibirica Ledeb. (Siberian larch) of a new flavan not previously described in the literature [1], giving a pink coloration with vanillin in concentrated HCl, which we called listvenol.

Listvenol, with the composition $C_{30}H_{24}O_{10}$, is a colorless crystalline substance with mp 210-212°C, $[\alpha]_D^{20}$ −152° (acetone–water), λ_{max} 276 nm readily soluble in alcohols, ethyl acetate, and acetone and moderately soluble in water and ether.

The alkaline cleavage of listvenol forms p-hydroxybenzoic and p-hydroxyphenylacetic acids and phloroglucinol, like the similar cleavage of 3,4',5,7-tetrahydroxyflavan [(−)-epiafzelechin], which is present in this plant. For listvenol the following structure has now been established:



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As compared with the spectrum of (−)-epiafzelechin (Fig. 1, a) the IR spectrum of listvenol (Fig. 1, b) has an additional absorption band at 1790 cm^{-1} which may belong either to an ester bond or to a β-pyrone or to a conjugated enol [2]. Since the substance does not undergo alkaline hydrolysis or specific enzymatic hydrolysis with tannase, it does not contain an ester bond. It does not give reactions for ketal groups. The presence of a double bond in it was confirmed by positive qualitative reactions of the peracetate with a solution of bromine in carbon tetrachloride and with potassium permanganate. A quantitative determination by Meyer's method [3] showed 100% of the enolic form.

The NMR spectrum of listvenol (Fig. 2, b) contains a one-proton singlet at 6.13 ppm which is shifted downfield in the octaacetate (6.24 ppm) and is due to a vinyl proton. The integral intensity of the latter confirms the presence of 100% of the substance in the enolic form.

The production of an octaacetate shows the presence of eight hydroxy groups in the substance. In the NMR spectrum of the octaacetyl derivative (Fig. 2, c) there are the signals of 18 protons of aromatic acetate groups at 2.16-2.34 ppm and singlets at 1.80 ppm, corresponding to the protons of an enolic acetate group, and at 1.70 ppm, due to the three protons of an alcoholic acetate group.

Methylation with diazomethane formed a heptamethyl ether, the acylation of which gave the heptamethyl ether of the monoacetate. The NMR spectrum of the latter contained the signal of three protons (1.70 ppm, singlet) of a single alcoholic acetate group and the signals of 21 protons (2.16-2.34 ppm) corresponding to methoxy groups. Consequently, the seven hydroxy groups out of the eight free hydroxy groups are methylated by diazomethane, which confirms the presence of six phenolic, one enolic, and one secondary alcoholic groups. The H-3 proton relating to the secondary alcohol group underwent a characteristic downfield shift from 4.15 to 5.16 ppm after acylation.

S. M. Kirov Kazakh State University. Translated from Khimiya Prirodnykh Soedinenii, No. 5, pp. 623-629, September-October, 1973. Original article submitted July 24, 1972.

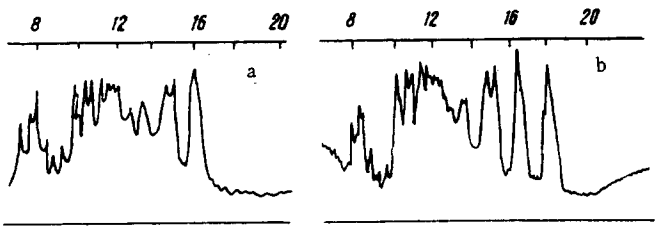
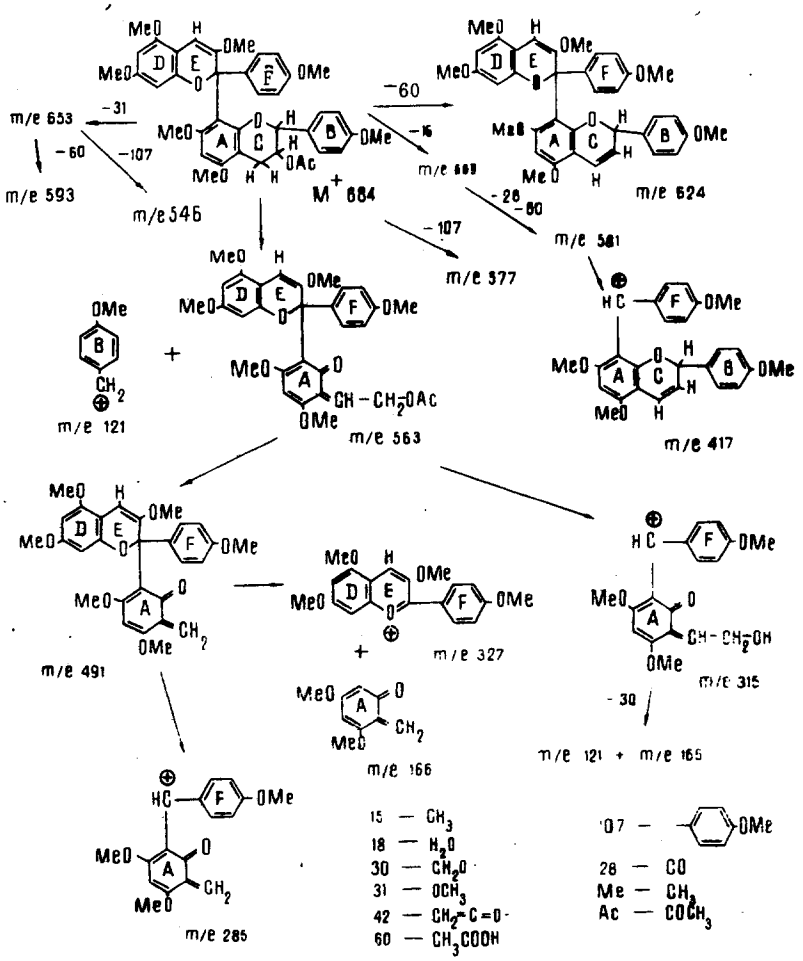


Fig. 1. IR spectra of (−)-epiafzelechin (a) and listvenol (b).

In the mass spectrum of the monoacetyl heptamethyl derivative (Fig. 3), there is the molecular peak with a mass number of M^+ 684, with a low intensity as a result of the rapid cleavage of the molecule at the moment of introduction. A fragment with m/e 624 (see scheme) arises from the molecular ion by the direct ejection of a molecule of acetic acid. The ease of ejection of acetic acid shows the acylation of a secondary alcohol group [4]. The diene cleavage of the catechin moiety of the molecule leads to the formation of fragments with m/e 563 and 491. The presence of fragments with m/e 417, 315, and 285 shows a strong 2-8 bond between the molecules.

Such a scheme of cleavage is also observed in the mass spectrum of the heptamethyl ether of listvenol.

Fragmentation of the monoacetyl heptamethyl derivative of listvenol



In the NMR spectrum of listvenol (see Fig. 2, b) there are the signals of the eight protons of two para-substituted benzene rings: a doublet at 7.02 ppm ($J_{2',3'}$ = 8 Hz) due to the four 2',6' protons, a doublet at 6.79 ppm ($J_{5',6'}$ = 8 Hz), corresponding to the two 3',5' protons of ring B and a doublet at 6.6 ppm ($J_{5',6'}$ = 8 Hz) corresponding to the two 3',5' protons of ring F. The paramagnetic shift of the 3',5' protons of ring F can be explained by the presence in it of a nucleophilic substituent (ring A). The signals of the protons of rings A and D are represented by two one-proton doublets at 5.98 and 6.03 ppm ($J_{6,8}$ = 2 Hz), which are

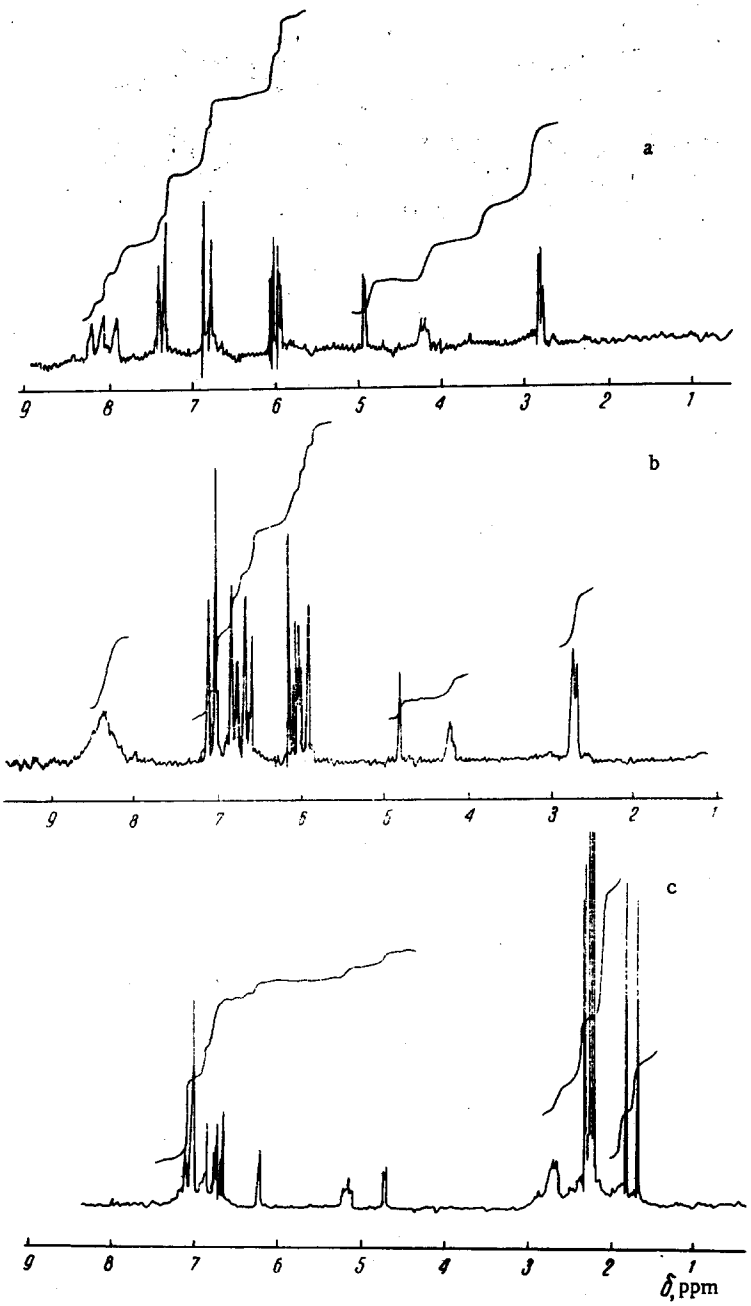


Fig. 2. NMR spectra of (−)-epiafzelechin (a), listvenol (b), and listvenol octaacetate (c).

characteristic for a meta-substituted ring, and a singlet at 5.85 ppm with an integral intensity of one proton. On comparing this NMR spectrum with that of (−)-epiafzelechin (see Fig. 2, a), it can be seen that the protons at 5.98 ppm and 6.03 ppm correspond to 6-H and 8-H of ring D and the singlet at 5.85 ppm corresponds to 6-H of ring A. This is also confirmed by the absence of splitting of the 6-H signal of ring A.

The protons of the heterocyclic rings are represented by one vinyl proton (narrow singlet at 6.13 ppm), one 3-H proton of a secondary alcohol group (multiplet at 4.15 ppm, $J_{2,3}=2$ Hz, $J_{3,4}=4$ Hz), one CH_2 group (doublet at 2.65 ppm, $J_{3,4}=4$ Hz), and one 2-H proton at 4.78 ppm, a broadened singlet because of the weak bond with the aromatic protons of ring B.

The results of a comparison of the 2-H signal with the signal of the corresponding proton of (−)-epiafzelechin and literature information [5] and also the splitting of the 3-H signal into a multiplet permit the deduction that this proton is present in ring C. On the basis of the $J_{2,3}$ value of 2 Hz, it may be concluded that the protons mentioned have the cis configuration [6].

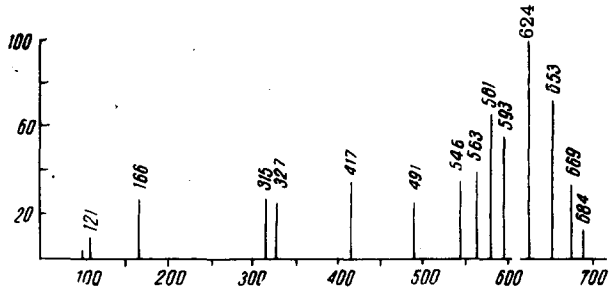


Fig. 3. Mass spectrum of the monoacetyl heptamethyl derivative of listvenol.

Thus, the absence of a proton on the C_2 atom of ring E and of a proton at the C_8 atom of ring A makes it possible to suggest that these carbon atoms take part in the bond between the molecules. This form of the bond is confirmed by its stability to heating with mineral acid.

Under mild conditions of hydrolysis (0.01 N HCl), no formation of catechin and anthocyanidin was observed; no anthocyanidin was formed, either, under more severe conditions (2 N HCl in methanol). Prolonged heating with an acid formed a brown phlobaphene. A similar type of bond and its resistance to acid in synthetic dimers of catechins has been shown in the work of Freudenberg and Weinges [7] and of Brown et al. [8].

The existence of natural flavonoids in the form of flav-3-enols has been suggested on theoretical grounds by Freudenberg and Weinges [9] and by King and Freudenberg [10]. Freudenberg and Weinges suggested the presence of a ketone group capable of passing into an enol in the dimer from gleditschin [11], and of a flavene grouping in the dimer from the cowberry [12]. However, these structures were not confirmed by more detailed investigations using physicochemical methods [13]. Thus, listvenol has proved to be the first representative of the natural flav-3-enols.

The native character of listvenol is shown by its detection in the ground bark directly after its removal from the tree. Listvenol is the main flavonoid of the bark of the Siberian larch. Its mean content in the bark is 0.1%.

Methylation of the flavonoid with diazomethane gave a hexamethyl derivative the acetylation of which formed a diacetate. As the NMR spectrum of the diacetyl hexamethyl derivative showed, the second acetate group is aromatic.

EXPERIMENTAL

The specific rotation was determined on a CM circular polarimeter. Thin-layer chromatograms were run on Silufol UV-254 (Czechoslovakia) in the chloroform–ethyl acetate (1:1) system and were viewed in UV light.

The IR spectra were taken on a UR-20 spectrometer (KBr tablets), and the NMR spectra on a Varian HA-100 D instrument [carbon tetrachloride and deuterated acetone; internal standards tetramethylsilane and hexamethyldisilazane; chemical shifts given in the δ scale], and the mass spectra on an MKh-1304 instrument [(modified) with a system of direct introduction, with heating; energy of the ionizing electrons 70 eV]. The results of the analysis of all the compounds corresponded to the calculated figures.

Separation of the Flavans. The carefully ground bark of Larix sibirica Ledeb. (10 kg) was treated with benzene to eliminate resins and then twice with acetone (a total of 30 liters). The combined acetone extracts were evaporated to dryness under a reduced pressure of nitrogen at 40°C. The residue was dissolved in aqueous sodium bicarbonate solution, and the flavonoids were extracted with ether. The dry ethereal extract was first washed with benzene and was then treated with chloroform–ethanol–water (8:2:1) [14] and was chromatographed on columns of cellulose. Fraction I contained flavonols, fraction II catechins, and fraction III listvenol contaminated with catechins. On standing for several days, fraction III deposited crystals of listvenol, which were purified by repeated rechromatography under the same conditions: $C_{30}H_{24}O_{10}$, mp 210-212°C (from ethanol–chloroform–water), $[\alpha]_D^{20}$ −152° [c 7.5; acetone–water (1:1)], R_f in BAW (40:12.5:29) (1) 0.83 and in 2% acetic acid (2) 0.38. Yield 5 g.

The separation of fraction II was performed by partition chromatography on KSK silica gel with ether as the eluent. Fraction I of the eluent contained (−)-epiafzelechin, the final purification of which was per-

formed by chromatography on Sephadex G-25 with water as the eluent, and this was followed by (+)-catechin and (−)-epicatechin [1]. (−)-Epiafzelechin, $C_{15}H_{14}O_5$, mp 252-253°C, $[\alpha]_D^{20}$ −58.8° [c 10.0; acetone−water (1:1)], R_f 0.70 (1) and 0.35 (2).

Alkaline Cleavage. A mixture of 10 mg of listvenol and 2 ml of 50% KOH was heated at 170°C for 20 min. Then it was neutralized with 25% sulfuric acid and extracted with ethyl acetate. In the cleavage products, phloroglucinol and p-hydroxybenzoic and p-hydroxyphenylacetic acids were identified in comparison with markers in various solvent systems [15, 16].

Octaacetyllistvenol. A mixture of 500 mg of listvenol, 5 ml of freshly distilled acetic anhydride, and 100 mg of calcined sodium acetate was heated in the boiling water bath for 4 h and at a gentle boil of the anhydride for 1 h. Then the reaction mixture was poured into cold water. The precipitate that deposited was filtered off, washed with water, and reacylated under the same conditions. Crystals from aqueous methanol, $C_{48}H_{40}O_{18}$, mp 121-123°C $[\alpha]_D^{20}$ −78° (c 4.5; acetone), R_f on TLC 0.68.

Methylation of Listvenol. A solution of 2 g of listvenol in 3 ml of absolute acetone was treated with a solution of diazomethane in ether for one day. The solvent was eliminated under vacuum, and the residue was washed with water and was remethylated with diazomethane under similar conditions. The crude product was dissolved in 25 ml of methanol, 30 ml of freshly distilled dimethyl sulfate was added, and an equivalent amount of 50% KOH solution was run in dropwise. Then the reaction mixture was heated in the water bath for 30 min and, after cooling, was poured into cold water. The precipitate that deposited was filtered off and washed with water.

The results of thin-layer chromatography showed that the product obtained contained two substances: (I) with R_f 0.83 and (II) with R_f 0.63. Separation was performed by repeated chromatography on columns of silicic acid/Chromaton (5:1). Substance (I) was eluted with petroleum ether and substance (II) with benzene.

Heptamethyl Ether of Listvenol; Substance (I). This crystallized from a mixture of petroleum ether and benzene (6:4); $C_{37}H_{38}O_{10}$, mp 180-182°C, $[\alpha]_D^{20}$ −184.2° (c 1.7; acetone).

Hexamethyl Ether of Listvenol; Substance (II). This crystallized from benzene−chloroform (1:1); $C_{36}H_{36}O_{10}$, mp 137-139°C, $[\alpha]_D^{20}$ −111.5° (c 5.3; acetone).

Monoacetyl Heptamethyl Derivative of Listvenol. The hexa- and heptamethyl ethers of listvenol were acylated under the conditions described above. From aqueous methanol the monoacetyl heptamethyl derivative formed colorless crystals, $C_{39}H_{40}O_{11}$, with mp 120-122°C, $[\alpha]_D^{20}$ −169° (c 1.67; acetone).

Diacetyl Hexamethyl Derivative of Listvenol. From aqueous methanol a white product was obtained with the composition $C_{40}H_{40}O_{12}$, mp 118-120°C, $[\alpha]_D^{20}$ −111.4° (c 2.3; acetone).

SUMMARY

A new polyphenol − listvenol − has been isolated from the bark of Larix sibirica Ledeb. Structure (I) is proposed for it on the basis of chemical and spectral characteristics.

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