Isolation of the Polysaccharide. After treatment with boiling 82% ethanol (1 h), the leaves were dried in the air and the polysaccharide mixture was extracted from them with water (65°C, 1 h). After dialysis against mains water and distilled water (7 days) and evaporation in a rotary evaporator, the polysaccharide mixture was precipitated with ethanol (1:4) and was freeze-dried. Then the individual polysaccharide was isolated from the mixture with the aid of DEAE-cellulose.

CONCLUSION

A polysaccharide has been isolated from the leaves of *Phytolacca americana* and has been characterized. It has been established that it contains residues of galactose, arabinose, xylose, and rhamnose in a ratio of 3:4:1:3 together with 85-90% of D-galacturonic acid residues. The results obtained permit the polysaccharide to be assigned to the class of pectin substances.

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NEW NATURAL PHENOLIC TRIGLYCERIDES

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UDC 547.915

The composition of the phenolic triglycerides isolated from ethanolic extracts of propolis, aspen buds, and wheat roots has been investigated. Two new phenolic triglycerides have been identified: 2-acetyl-1,3-diferuloylglycerol, and 2-acetyl-3-p-coumaroyl-1-feruloylglycerol.

Recently, new natural triglycerides substituted in positions 1 and 3 by residues of cinnamic acid and its derivatives were isolated from the buds of *Populus lasiocarpa* [1, 2]. Later, glycerides of this type were also found in the fruit of *Aegilops ovata* L. [3], and in these the hydroxyl group in position 2 remained unsubstituted.

We have also determined a fraction containing phenolic glycerides in ethanolic extracts of the buds of another species of poplar, *Populus tremula* L. (aspen), and also in the propolis which honeybees collect from the secretions of its axillary buds [4]. Since phenolic triglycerides are present in the buds themselves in extremely minute amounts, we used the more accessible propolis of the appropriate type. The triglyceride fraction of this product was isolated by chromatographing the dry residue of an ethanolic extract on columns of silica gel in a n-heptane–ethyl acetate gradient system. The isolation was monitored by TLC on Silufol. The phenolic triglycerides had R_f 0.7 in the benzene–ethyl acetate–methanol (10:4:1) system and were readily detected from the nature of their fluorescence in UV light or from the pinkish color of the spots when the plates were sprayed with concentrated H_2SO_4. The subsequent purification of the triglyceride fraction from the phenols accompanying it was performed by acetylation with Ac_2O in pyridine. The product formed, after the usual working up, was separated on preparative plates coated with silica gel by two runs of the ethyl acetate–n-heptane (1:2) system.

Below, we give the results of a mass-spectrometric analysis of the acetylated friction of the phenolic triglycerides from propolis:

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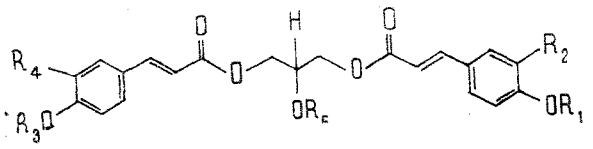
Compound	$[M]^+$		$[M-CH_2CO]^+$		$[M-2CH_2CO]^+$	
	m/z	*J*	*m/z*	*J*	*m/z*	*J*
II	570	2	528	44	486	42
V	540	1	498	8	456	3
VIII	510	1	468	4	426	3

According to the results of mass spectrometry, the chromatographically homogeneous fraction obtained included several closely related compounds having m/z values of the M^+ ions of 570, 540, and 510, respectively. The peaks of these ions were of low intensity, but high-intensity peaks of the ions formed as the result of the ejection from the initial molecules of an acetyl residue (in the form of $CH_2=C=O$) corresponded to them (see above), which is characteristic for compounds of the type mentioned [1, 2]. In particular, to the main compound of this fraction with m/z 570 for M^+ ion corresponded intensive ejections with the formations of ions having m/z 528 and 486.



I. $R_1=R_3=H$, $R_2=R_4=OCH_3$, $R_5=COCH_3$
II. $R_1=R_3=R_5=COCH_3$, $R_2=R_4=OCH_3$

III. $R_1=R_3COC^2H_3$, $R_2=R_4=OCH_3$, $R_5=COCH_3$
IV. $R_1=R_3=R_5=H$, $R_2=R_4=OCH_3$
V. $R_2=OCH_3$, $R_1=R_3=R_4=H$, $R_5=COCH_3$
VI. $R_2=OCH_3$, $R_4=H$, $R_1=R_3=R_5=COCH_3$
VII. $R_2=OCH_3$, $R_4=H$, $R_1=R_3=COC^2H_3$, $R_5=COCH_3$
VIII. $R_1=R_2=R_3=R_4=H$, $R_5=COCH_3$
IX. $R_2=R_4=H$, $R_1=R_3=R_5=COCH_3$
X. $R_2=R_4=H$, $R_1=R_3=COC^2H_3$, $R_5=COCH_3$

The amount of this substance in the fraction studied rose considerably after additional chromatographic purification by the TLC method in the above-mentioned system and then in the benzene–ethyl acetate (3:1) system, where the glyceride had R_f 0.7. In the NMR spectrum of the substance obtained, taken in C^2HCl_3 solution, three-proton singlets of three acetoxy groups were clearly revealed, two of which had chemical shifts of 2.24 ppm and one of 2.08 ppm, and also the signals of two methoxy groups at 3.86 ppm. The spectrum also included the signals of six aromatic protons forming an unresolved multiplet at 7.06 ppm and of two two-proton doublets at 6.46 and 7.64 ppm with J = 16 Hz, which corresponds to the presence of two disubstituted Ar–CH=CH–CO-trans groups in the molecule. The NMR spectrum also contained the signals of the protons of a substituted glycerol residue: one-proton and four-proton multiplets at 5.3 and 4.36 ppm, respectively, similar to those described previously for 1,3-diaroyl-2-acylglycerol [2].

It follows from these facts that the acetate under consideration is an acetyl derivatives of a diferuloylglycerol. The NMR spectrum of acetylferulic acid measured in the same solvent showed analogous signals, namely: two-proton doublets of a –CH=CH=CO group at 6.39 and 7.64 ppm with J = 16 Hz and three-proton singlets at 3.83 and 2.29 ppm for OCH_3 and $OCOCH_3$ groups, respectively.

The proposal of structure (II) for the acetate of the product isolated agrees well with the results of high-resolution mass spectrometry. The molecular ion of this substance had the composition $C_{29}H_{30}O_{12}$ (measured 570.1727; calculated 570.1726) and the ions due to the ejection of acetyl groups had the respective compositions $C_{27}H_{28}O_{11}$ (measured 528.1604; calculated 528.1576), and $C_5H_{26}O_{11}$ (measured 476.1516; calculated 486.1515). The mass spectrum of the acetate also has characteristic high-intensity peaks of ions due to the presence of a ferulic acid residue in the molecule including those with the composition $C_{10}H_{10}O_4$ [(OH)(OCH_3)–C_6H_3–CH=CH–COOH; measured 194.0579; calculated 194.0579] and $C_{10}H_9O_3$ [(OH)(OCH_3)–C_6H_3–CH=CH–CO^+; measured 177.0549; calculated 177.0546]. Similar ions were found previously for compound (IX) [1].

To confirm the structure of the acetate isolated, it was synthesized from acetylferulic acid chloride and glycerol in the presence of pyridine under the conditions previously de-

scribed [2], followed by acetylation of the product formed. The acetate obtained proved to be completely identical in its R_f values and NMR and mass spectra with the acetyl derivative that we had isolated and also to the compound obtained by acetylating natural 1,3-diferuloylglycerol (IV) [3].

To determine the substitution in position 2, the natural triglyceride fraction was acetylated by the action of $(C^2H_3CO)_2O$ in pyridine. The NMR spectrum of the trideuteroacetate (III) formed showed a three-proton singlet signal of one acetoxy group at 2.08 ppm but there were no similar signals in the weaker field characteristic for aromatic CH_3CO groups [2]. Consequently, in the initial substance the acetoxy group at C_2 is a natural substituent and the substance is the previously undescribed 2-acetyl-1,3-diferuloylglycerol (I).

Analysis of the mass spectrum of the product of the acetylation of the initial fraction of phenolic triglycerides isolated from the same source showed the presence in it of two other compounds of similar nature. The m/z values of their molecular and fragmentary ions indicated that they were di-p-coumaroyl- and p-coumaroylferuloylglycerols. For the latter compound, the strongest fragmentary ion, $(M - CH_2CO)^+$ with m/z 498, had the composition $C_{26}H_{26}O_{10}$ (measured 498.1518; calculated 498.1515). The structures of these glycerides as (V) and (VII) were confirmed by analysis of the products of trideuteroacetylation — compounds (VII) and (X), respectively. In their mass spectra, the m/z values of their M^+ ions were 546 and 516, i.e., shifted by 6 a.m.u. in the direction of higher masses. To these ions corresponded fragmentary ions with m/z 502 and 548 for compound (X) and 472 and 428 for compound (VII), obviously formed by the ejection of a molecule of deuteroketene (C^2H_2=C=O), the ratio of the intensities of these ions being approximately the same as for the triglyceride acetate (III). This means that in these molecules the first acetyl group is natural and is obviously located at C_2 of the glycerol residue. In actual fact, the component with a M^+ ion having m/z 510 has previously been detected in the buds of *P. lasiocarpa* [2] and its characteristic fragmentary peaks (468, 305, 189, 147, 119, 91, 65, and 43) were also readily traced in the product of the acetylation of the mixture under investigation. In the spectrum of the second component, peaks of ions characterizing the simultaneous presence in it of residues of ferulic and p-coumaric acids (498, 456, 219, 189, 147) were observed and, consequently, it is the previously undescribed 2-acetyl-3-p-coumaroyl-1-feruloylglycerol (V).

By making use of a similar method of isolation, we have analyzed the buds of *Populus tremula* and also wheat roots for the presence of phenolic triglycerides (see below).

As we expected, the composition of the triglyceride fraction of aspen buds proved to be close to that of the fraction of propolis that we studied, but, in contrast to the latter, the mixed triglyceride (V) predominated in it. These differences are possibly due to the fact that for this analysis we used wintering aspen buds, while bees collect propolis from the secretions of summer axillary buds [4]. In actual fact, as we have established previously [5], the composition of the phenylpropanoid compounds in these organs varied considerably according to the season.

In wheat roots the main component in the fraction of phenolic triglycerides proved to be the diferulate (IV). This compound has an unsubstituted hydroxy group at C_2 of the glycerol residue, as is shown by the mass spectrometry of its trideuteroacetyl derivative: a displacement of the m/z value of its M^+ ion by 9 a.m.u. in the direction of higher masses (from 570 to 579). The same substance was previously isolated from *Aegilops ovata* [3], which confirms the assumption of a phylogenetic affinity of these species.

Thus, the results obtained indicate that phenolic triglycerides are widespread in various natural sources, including those having medicinal and food value.

Compound	Propolis	Populus tremula	Triticum sativum	Aegilops ovata [3]
I	+	+	—	—
IV	—	—	+	+
V	+	+	—	—
VIII	+	+	—	—

Attention is attracted by the fact that according to Asakawa et al. [2], these compounds are not cleaved by human lipase and therefore they can pass directly into the blood circulation of the organism.

EXPERIMENTAL

Propolis of the aspen type were collected in the apiary of the Moscow breeding station (village of Uzunovo, Serebryanye Prudy district), and the aspen buds in the environs of the village of Podlipki in the same region. Wheat roots of the variety Mironovskaya 808 were dug up in 1979 season from the seed-stock plots of the Moscow breeding station.

The mass spectra of the compounds were recorded on a LKB-9000 instrument at a temperature of 100-120°C with an ionization potential of 70 V. High-resolution spectra were recorded on a Varian MAT-44 instrument and NMR spectra on a Varian XL100 instrument. Deuterochloroform was used as the solvent with TMS as internal standard. To synthesis compound (II) we used ferulic acid from Serva (USA). The ethyl ester of the acetate of this acid, which was necessary for measuring the NMR spectrum, was obtained by the method of Allen and Gyers [6].

Isolation of the Phenolic Triglycerides. A. Propolis that has been cooled to 0°C and ground into a powder in an electric mill (28 g) was extracted with 500 ml of ethanol for a day. The mixtures was filtered and the filtrate was evaporated in vacuum. The residue (21 g) was dissolved in chloroform, 50 g of silica gel L 100-160 μ (Czechoslovakia) was added, and the mixture was evaporated to dryness in vacuum. The residue obtained was found in a mortar and was transferred to the top of 70 × 100 mm column filled with the same absorbent in n-heptane solution. Elution was carried out with a n-heptane–ethyl acetate gradient system. Separation was monitored by the TLC method on Silufol UV_{254} plates in the benzene–ethyl acetate–methanol (10:4:1) system.

The fraction having as their main components substances with R_f 0.7-0.75 were combined and evaporated, and the residue (150 mg) were separated into two equal parts, one of which was acetylated by the action of acetic anhydride in pyridine while the other was subjected to the action of [D]acetic anhydride in the same solvent. The two solutions were left overnight, after which each of the reaction mixtures was diluted with water, transferred to a separating funnel, and extracted with ethyl acetate (2 × 20 ml). The organic layer was separated off and was washed successively with dilute HCl (2 × 20 ml), 5% $NaHCO_3$ (2 × 20 ml), and with water and was dried over $MgSO_4$ and evaporated to dryness in vacuum. The acetyl and [D]acetyl fractions so obtained were subjected to repeated preparative TLC on 20 × 20 cm plates coated with silica gel LL_{254} 5-40 μ in the n-heptane–ethyl acetate (2:1) system.

Zones with R_f 0.2 having a characteristic dark violet fluorescence under UV light and a peak coloration when the plates were sprayed with concentrated H_2SO_4 were cut out, the adsorbent was scraped off, and it was eluted with chloroform (2 × 50 ml), after which the extract was evaporated in vacuum to dryness. This gave 40 mg of each fraction, and the material was additionally purified by PTLC on 20 × 20 cm plates coated with LL_{254} 5-40 μ silica gel in the benzene–ethyl acetate (3:1) system.

The fraction with R_f 0.7 was cut out and eluted with chloroform (2 × 20 ml), and the chloroform extracts were dried in vacuum. This gave 30 mg of each of the fractions containing the products of the acetylation of the phenolic triglycerides.

B. Aspen buds (250 g) were extracted with 500 ml of methanol for three days. The extract obtained was filtered and evaporated in vacuum. The residue (22 g) was subjected to purification as described in paragraph A.

C. Freshly dug-up wheat roots (3 kg) were carefully washed free from soil with cold water and were ground and extracted with 6 liters of methanol for three days. The extract was filtered and evaporated in vacuum. The dry residue (32.5 g) was purified as described in paragraph A. This gave 1.8 mg of acetyl and [D]acetyl derivatives of the phenolic triglyceride fractions.

The synthesis of 2-acetyl-1,3-diferuloylglycerol was carried out under the conditions described previously [2]. For this purpose, 6 ml of pyridine and 4 ml of $SOCl_2$ were added to 0.5 g of ferulic acid acetate, and the mixture was boiled until the acid had dissolved, after which 5 ml of freshly distilled $CHCl_3$ and 5 ml of absolute pyridine were added. With cooling to 0°C and with stirring, 1 g of glycerol was added, after which the reaction mixture was kept at 20°C for 12 h and was boiled under reflux for 4 h. After cooling, it was poured into water, and the aqueous layer was acidified with dilute HCl and extracted with ethyl acetate. The organic layer was washed with HCl solution, with water, with 5% $NaHCO_3$, again with water, dried over $MgSO_4$, and evaporated in vacuum to dryness. The residue so obtained was acetylated and the triglyceride fraction was isolated as described in paragraph A. This gave 50 mg of substance (II).

CONCLUSION

The composition of the phenolic triglycerides isolated from ethanolic extracts of propolis, aspen buds, and wheat roots has been investigated.

Two new phenolic triglycerides have been identified: 2-acetyl-1,3-diferuloylglycerol and 2-acetyl-3-p-coumaroyl-1-feruloylglycerol.

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A STUDY OF THE COUMARINS OF *Haplophyllum obtusifolium*. THE STRUCTURE OF OBTUSIDIN AND OF OBTUSIPRENIN

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The epigeal part of *Haplophyllum obtusifolium* Ledeb has yielded scopoletin and the following new coumarins: obtusidin, $C_{15}H_{16}O_5$, mp 165-167°C, and obtusiprenin, $C_{15}H_{16}O_5$, mp 139-140°C. It has been established on the basis of chemical and spectral characteristics that obtusidin has the structure of 3-(1'-1'-dimethylallyl)-7,8-dihydroxy-6-methoxycoumarin, and obtusipernin that of 5-(3',3'-dimethylallyl)-7,8-dihydroxy-6-methoxycoumarin.

Previously, we [1, 2] and other [3] workers have isolated a number of coumarins from *Haplophyllum obtusifolium* Ledeb. Conttinuing work in this direction, we have studied the coumarins of the epigeal part of the plant collected on the Ustyurt plateau in the fruit-bearing period on August 16, 1981. The ground raw material was repeatedly extracted with ethanol. The concentrated ethanolic extract was chromatographed on a column of silica gel, as a result of which we isolated 7-(3',3'-dimethylallyloxy)-6-methoxycoumarin, obtusinin, obtusoside [1], fraxetin, capensin, obtusicin [2], and another three coumarins not previously detected.

Coumarin (I) with mp 201-203°C was identified by its spectral characteristics and by a direct comparison with an authentic sample as scopoletin.

Coumarins (II) with mp 165-167°C, and (III), with mp 139-142°C proved to be new, and we have called them obtusidin and obtusiprenin, respectively. The two substances have the same composition $C_{15}H_{16}O_5$, M^+ 276. According to their IR spectra and a positive reaction with a solution of $FeCl_3$, coumarins (II) and (III) contain phenolic hydroxy groups. The PMR spectrum of (II) contains the signals of protons due to the presence of CH_3O (3.72 ppm), H-5 (6.58 ppm, s), H-4 (7.50 ppm, s), and a 1',1'-dimethylallyl grouping [4-7]. The absence of substituent at C-5 was confirmed by the value of the chemical shift of H-4 [7, 8]. Consequently, obtusidin is a 3,6,7,8-tetrasubstituted coumarin and contains two phenolic hydroxy groups.

When it was treated with acetic anhydride in pyridine, obtusidin formed a diacetyl derivative (IX) (2.27 and 2.33 ppm, 3 H each, 2 Ar-$OCOCH_3$, in the PMR spectrum). The formation of a methylenedioxy derivative (V) as the result of the reaction of (II) with CH_2I_2

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