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AN INVESTIGATION AND IDENTIFICATION OF POLYSACCHARIDES ISOLATED FROM ARCHEOLOGICAL SPECIMENS

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Carbohydrates may be preserved undisturbed for millions and thousands of millions of years in sedimentary rocks and fossilized plant and animal residues [1-5]. Of the polysaccharides in such specimens it is usually possible to determine cellulose, chitin, and starch [1-4], but it is not known how long the polysaccharides of other groups, especially gums, remain undisturbed. Plant gums were widely used in antiquity for preparing pigments [6], and therefore the investigation of an extremely unusual material – polysacchardies isolated from fragments of paintings – will enable us to determine whether gums remain unchanged for long periods. In the isolated experiments performed up to the present time, it has not been possible to identify the polysaccharides found in ancient paintings [7, 8].

Figure 1 shows the IR absorption spectra of compounds isolated from specimens of wall paintings of the following Central Asian buildings: a) Karatepe, second-fourth centuries; b) Pendzhikent, seventh-eighth centuries, c) Adzhinatepa, seventh century; d) Toprakkal, third-fourth centuries; e) Khiva, 19th century. A comparison of the spectra has shown that they are all very similar and consist of typical polysaccharide spectra. Thus, in the 3400-cm^{-1} region there is a broad and strong band of the stretching vibrations of bound OH groups, and at 2800-3000 cm^{-1} the band of the stretching vibrations of CH groups. Two bands are observed in the 1740 and 1620-1640 cm^{-1} regions in all the spectra. The first includes a band due to the stretching vibrations of C=O groups of unionized acids (some authors assign them to hydrate water [8]) and in the second there is the stretching vibration of the carbonyls of ionized acids forming complexes with inorganic ions [9, 10]. In the 1400-1450 cm^{-1} region there is the band of the planar deformation vibrations of CH groups, but bands corresponding to the vibrations of a carboxylate ion may also present here [10]. The stretching vibrations of the skeleton of the molecule are observed in the 1000-1100 cm^{-1} region. The difference of the spectra in this region is probably due to inorganic impurities and, in particular, to the presence of some traces of gypsum (particularly the absorption at $\sim$ 1000, 1120, and 1140 cm^{-1}) [11].

The characteristic nature of the features of the spectra for polysaccharides [7, 8, 10] emphasizes their distinct similarity to the IR spectrum of the gum of the apricot Armeniaca vulgaris (the gum was collected in the town of Tashkent) which is also given in Fig. 1f. The spectra of the gums of the sour cherry Prunus cerasus, of the mazzard cherry (Prunus avium), and of the apricot proved to be completely identical, and therefore it is impossible to distinguish the gums solely on the basis of a comparison of IR spectra.

The similarity of compounds *a* and b was also confirmed by a study of their composition by paper and thin-layer chromatography (Table 1). The same main components – xylose, mannose, and glucose – were detected in the two polysaccharides. In gas–liquid chromatography (GLC) of derivatives of a hydrolyzate of compound b it was found that it included, in addition to the monosaccharides already mentioned, considerable amounts of galactose and glucuronic acid. Consequently, in the cases described the gums of similar, but not identical, plants were apparently used. Since the spectra of the saccharides mentioned resemble the spectra of the gums of fruit trees (f) and are given in the literature [7], hydrolyzates of sour cherry and apricot gums were also analyzed by the GLC method. The compositions of these gums proved to be very similar (see Table 1), but the

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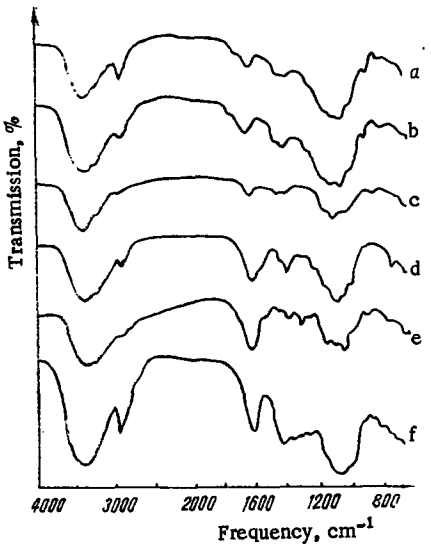

Fig. 1. IR spectra of polysaccharides isolated from paintings (*a*-e), and of apricot gum (f).

relative amounts of arabinose and galactose differed substantially: in a hydrolyzate of apricot gum the ratio of the monosaccharides was 3.0 : 1.0, and in sour-cherry gum it was 2.3 : 1.0 (in the literature, figures of 1.0 : 1.0 and 1.1 to 3.0 : 1.0, respectively, have been given [12-14]). These ratios were calculated approximately from the areas of the peaks of derivatives of the monosaccharides on chromatographs. Thus, the compositions of polysaccharides *a* and b are not similar to those of sour cherry and apricot. At the same time, the gums of some trees of the genus Prunus consist of xylose residues to the extent of more than 90% [12], and therefore it is not excluded that polysaccharides *a* and b do nevertheless represent components of the gums of certain trees of the family Prunoideae.

Product c is also a polysaccharide, as is shown by its IR spectrum. When a hydrolyzate of this polysaccharide was chromatographed on paper, glucose, xylose, and mannose were identified (see Table 1), the glucose spot having the strongest coloration. In this case, also, it is difficult to state which plant provided the gum used for the paintings.

The spectra of polysaccharides d and e are particularly similar to the spectrum of apricot gum. The stronger absorption bands in the 1400-1420 and 1620-1640 cm^{-1} regions in the spectra of these polysaccharides are probably due to the presence of dissociated carboxyl ions [10], and the band at 1240 cm^{-1} to the presence of inorganic components. When hydrolyzates of these polysaccharides were chromatographed in a thin layer of cellulose, the monosaccharides present in fruit-tree gums were found (see Table 1). Consequently, it can be stated that substances d and e most probably consisted of sour-cherry or apricot gum.

Thus, it is obvious that the polysaccharides of plant gums are capable under certain conditions of remaining largely undestroyed. This is shown by the similarity of the composition and properties of the polysaccharides *a* and b, and also of d and e. In the first case, the polysaccharides were isolated from fragments of the second-fourth and seventh-eighth centuries, and in the second case from the third-fourth and 19th centuries. Polysaccharides a and b possess similar properties: In addition to the similarity of their IR spectra, both substances are poorly soluble in water and they cannot be freed from inorganic impurities, with which they possibly form complexes. Likewise, the properties of the polysaccharides d and e are similar. In particular, they are readily soluble in water in spite of the fact that the age of one compound is more than 1500 yr and that of the other only 100 yr. The investigation of the composition of the polysaccharides isolated has shown that in some cases it is possible to state from which plant gum the particular polysaccharide derived. All the results mentioned once more emphasize the stability of carbohydrates that has been reported by workers in the analysis of palaeontological and geological specimens [1-5].

EXPERIMENTAL

The polysaccharides were isolated in the following way. A fragment of a painting was ground, the resulting powder was boiled with water for 10 min and the mixture was centrifuged, and the solid matter was treated successively with 1 N HCl and 1 N NaOH. All these solutions were combined, brought to neutrality, and dialyzed against water for 72 h. The dialyzate was evaporated in a rotary vacuum evaporator, and the IR spectra of the substances obtained were recorded on a Perkin–Elmer 257 instrument, 1 mg of the sample being moulded into a tablet with 150 mg of KBr.

TABLE 1. Compositions of Polysaccharides Isolated from Archeological Samples and from Apricot and Sour-Cherry Gums

Polysaccharide	Arabinose	Rhamnose	Xylose	Mannose	Glucose	Galactose	Glucuronic acid
a			+	+	+, tr.		
b			+	+	+	+	+
c			+	+	+		
d	+, tr.			+	+	+	
e	+			+	+	+	
Apricot gum	+	+	+	+	+	+	+
Sour-cherry gum	+	+	+	+	+	+	+

The polysaccharides (samples weighing 1-5 mg) were hydrolyzed in 1 N H_2SO_4 on the boiling-water bath for 10-12 h [15]. The hydrolyzates were neutralized with $BaCO_3$ and were investigated by descending paper chromatography in the butan-1-ol–pyridine–water (6 : 4 : 3) system [16] or in a thin layer of microcrystalline cellulose (GDR) with three runs at 37°C in the ethyl acetate–pyridine–water (100 : 35 : 25) system [17]. Aniline hydrogen phthalate was used to reveal the reducing sugars.

Gas–liquid chromatography was performed on a Pye Argon Chromatograph using a column containing 3% of EC NSS-M on Gas-Chrom Q at 180°C with argon as the carrier gas; 5 μl of a 20% solution in chloroform was introduced. The monosaccharides of the hydrolyzates were previously reduced to polyols and converted into acetylated derivatives [18]. To the dried, neutralized hydrolyzate of the polysaccharide (sample weight 2 mg) was added 10-15 mg of sodium tetrahydroborate in methanol, and the mixture was stirred and was left for 1 h. Then the excess of tetrahydroborate was decomposed with KU-2 cation-exchange resin (H^+), the solution was evaporated, the residue was treated with methanol and the methanol was evaporated off several times, and the final residue was carefully dried. After this, a mixture containing equal amounts of acetic anhydride and pyridine was added and the reaction system was left for 4 h. (In the performance of the gas-chromatographic separation of the hydrolyzates of the polysaccharides the authors were assisted by A. I. Usov.)

SUMMARY

Polymeric compounds have been isolated from archeological specimens (fragments of Central Asian paintings of the 2nd-19th centuries). A comparison of their IR spectra with the spectra of plant gums has shown that they are polysaccharides which have remained undestroyed for up to 1800 yr. In some cases it has been possible from the IR spectra and composition of the extracted polysaccharides to provisionally identify sour-cherry or apricot gum, of which these polysaccharides were components.

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COMPOSITION OF THE PHOSPHOLIPIDS OF Gossypium barbadense

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We have previously [1] reported the isolation of the total phospholipids from the seed kernels of the fine-fibered cotton plant of variety 5904-I by Folch's method [2] and their purification by precipitation with acetone. The phospholipids obtained in this way amounted to 1.6% on the absolutely dry kernels, and the amount of phosphorus in them was 2.4%.

Continuing an investigation of these phospholipids, we have determined their qualitative composition by one-dimensional chromatography in a thin layer of silica gel in system 1 [3]. The chromatogram showed 10 spots of substances which were identified by chemical tests [4-5] and by comparison with markers; the R_f values of the substances were: 1 – 0.96; 2 – 0.92; 3 – 0.80; 4 – 0.78; 5 – 0.75; 6 – 0.55; 7 – 0.40; 8 – 0.20; 9 – 0.10; and 10 – 0.05. Six spots (1, 3, 5, 6, 7, and 8) gave a positive reaction for phosphorus [5]. We identified these substances as X_1-polyglycerophosphatides (1), X_2-polyglycerophosphatides (3),* phosphatidylethanolamines (5), phosphatidylcholines (6), phosphatidylinositols (7), and lysophosphatidylcholines (8).

After the chromatograms had been sprayed with sulfuric acid and slowly heated, the substances with R_f 0.92 and 0.78 gave a violet color, and then carbonized to black. These substances were isolated preparatively by chromatography on a column of silica gel and were characterized qualitatively as steroids [6]. The substances with R_f 0.10 and 0.05 were identified as carbohydrates, and visually they amounted to a considerable part of the sample studied.

The results of the investigation performed showed the complexity of the composition of the total material isolated, which contained as accompanying components, in addition to the phospholipids, two other groups in each case – steroids and carbohydrates. The presence of carbohydrates made further experiments difficult, since they interfered with the separation of the total phospholipids into individual components by column chromatography on silica gel.

The most widely used method of purifying lipid extracts from nonlipid impurities is that given in our first paper [1]. In this method, water and aqueous solutions of salts are used for washing, but this is most effective for lipid extracts of animal tissues containing only slight amounts of water-soluble impurities. Nonlipid components can also be separated by gel filtration on Sephadexes G-25 [7-10] and LH-20 [11, 12].

To free the total phospholipids from carbohydrates we used Molselekt G-25. For 1 g of dry gel we used 100-125 mg of total phospholipids. After the gel had been swollen in a mixture of chloroform, ethanol, and water (90 : 10 : 1) [10], it was charged into a column, and then the sample was deposited in the same mixture of solvents and it was eluted. The completeness of the filtration of the phospholipids was checked in a thin layer of silica gel in system 1. Purification from carbohydrates was complete, and the yield of phospholipids was 1.0% on the absolutely dry kernels and 68.8% on the impure material applied to the column; its phosphorus content had risen to 4.15%. The Molselekt used was discharged from the column, and the carbohydrates were re-

*For X_1- and X_2-polyglycerophosphatides, see the following paper.

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