COMPOSITION OF THE COMPLEX OF BIOPOLYMERS OF THE CELL WALLS OF THE GRAPE

E. N. Datunashvili, V. Ya. Chirva, V. N. Ezhov,
V. I. Rcheulishvili, and G. V. Vel'mizeva

UDC 581.192+634.8

The complex of high-molecular-weight substances of the cell walls of the grape has been studied. It has been shown that the complex contains polysaccharides, proteins, and phenolic substances presumably linked through O-glycosidic bonds.

In spite of the large number of studies in which the high-molecular-weight compounds of the skin of the grape have been considered [1], the complex form of these compounds have been studied inadequately [2], particularly against the background of other plant materials [3]. Nevertheless, the complex of biopolymers of the cell walls of the grape plays an important role in the processes of its ripening, and in the industrial processing of grapes it affects the intensity of extraction of the juice and the filterability and future colloidal stability of the wines [4].

We have studied the composition of the complex of high-molecular-weight substances of the cell walls (CWs) of grapes of the variety Saperavi. The isolation and purification of the CWs was carried out by the scheme given below (EIS — ethanol-insoluble preparation of the skins; UCWs — undelignified cell walls; DCWs — delignified cell walls): (See scheme on next plate.)

As a result of fractionation, the initial preparation of the skins (EIS), CW preparations with intermediate degrees of purification (CW-1 to CW-4), and purified preparations (CWs_0) containing and not containing lignin were obtained. The qualitative compositions of the fractions isolated and the quantitative amounts of the individual components in them were established, after acid and alkaline hydrolyses, by gas—liquid, thin-layer, ion-exchange, and paper chromatography (Tables 1-3).

The main component of the CWs of grape skins consists of polysaccharides (44.3-45.5 wt.%), while structurally bound protein and phenolic substances were detected in the smaller amounts (0.72-3.39% and 0.29-1.06%, respectively). The successive purification of the EIS was ac-

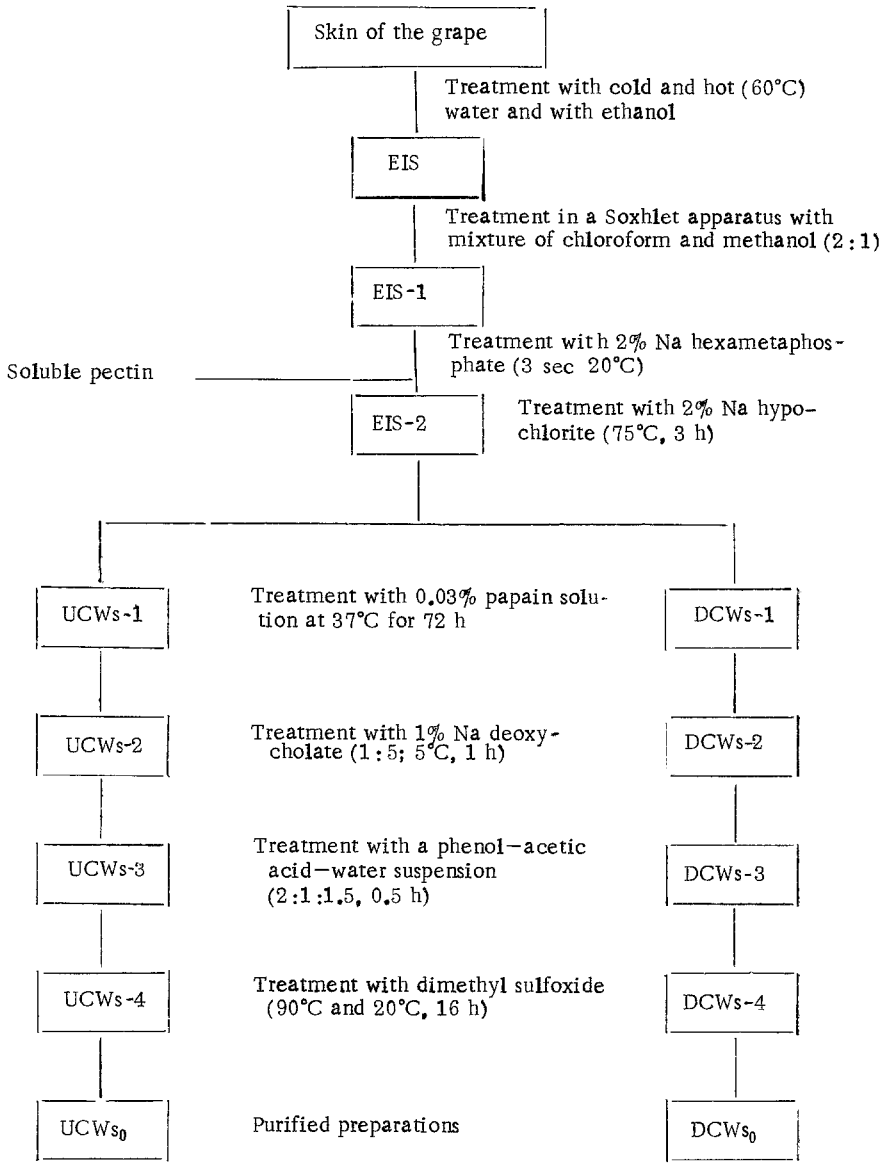
TABLE 1. Composition of the Polysacharides of Grape Cell Walls

Preparation, fraction after hydrolysis		Monosaccharide, wt.%					
		D-galacturonic acid	D-glucose + D-galactose*	D-mannose	L-arabinose	D-xylose	sum
EIS	Readily hydrolyzable (RH)	2,9	19,0	Tr.	3,1	1,7	26,7
	Difficultly hydrolyzable (DH)	0,6	9,6	5,7	—	2,6	18,5
$UCWs_0$	RH	—	11,6	2,6	2,0	3,9	20,1
	DH	0,4	17,9	4,7	—	1,2	24,2
$DCWs_0$	RH	Tr.	6,4	1,1	2,6	3,7	13,8
	DH	—	25,9	4,2	—	1,6	31,7

*The difficultly hydrolyzable fractions contained only glucose.

"Magarach" Scientific-Research Institute of Wine-Making and Wine-Growing, Yalta. M. V. Frunze Simferopol' State University. Khimiya Prirodnykh Soedinenii, No. 5, pp. 564-567, September-October, 1984. Original article submitted September 6, 1983.

 0009-3130/84/2005- 0530$08.50



companied by a redistribution of the amounts by weight of readily and difficultly hydrolyzable polysaccharides in the direction of a predominance of the latter; this feature was more pronounced for the delignified fractions.

The protein part of the EIS consisted of 13 amino acids in a total amount of 0.72%. The subsequent fractionation of the preparation led to an increase in the percentage of protein; a distinguishing feature of the purified CWs was the presence of a considerable amount of hydroxy amino acids (serine, threonine), while the $UCWs_0$ preparation also contained phenylalanine and tyrosine.

The main representatives of the phenolic substances in the CWs were phenolic carboxylic acids, which were more characteristic for the lignin-containing preparation. With the exception of p-hydroxybenzoic acid, they all had reactive hydroxy or methoxy groups.

The constant presence in the CW preparations, regardless of the degree of their purification, of protein and phenolic components indicates the formation of strongly bound protein-carbohydrate-phenol components in the skin. The presence in them of hydroxy compounds (hydroxy amino acids and hydroxyphenolic acids), arabinose, and galactose indicates their possible linkage through O-glycosidic bonds, by analogy with the complexes of other plant material [5].

EXPERIMENTAL

The acid hydrolysis of the polysaccharides was carried out in two stages, with 2% HCl (100°C, 3 h) and with 72% H_2SO_4 (20°C, 8 h; dilution to a concentration 5%, and boiling at

TABLE 2. Composition of the Proteins of Grape Cell Walls

Amino acid	Amount in the preparation, wt. %			Amino acid	Amount in the preparation wt. %		
	EIS	$UCWs_0$	$DCWs_0$		EIS	UCWs.	DCWs.
Aspartic acid	0,02	0,19	0.31	Valine	0,09	0,3	0,3
Threonine	Tr.	0,17	0,16	Methionine	Tr.	Tr.	—
Serine	Tr.	0,23	0,21	Isoleucine	0,09	0,27	0,27
Glutamic acid	0,09	0,53	0,59	Leucine	0,13	0,51	0,55
Proline	0,17	0,18	0,18	Tyrosine	Tr.	0,19	0,02
Glycine	0,05	0,21	0,29	Phenylalanine	0,03	0,3	0,08
Alanine	0,05	0,31	0,21	Sum	0,72	3,38	3,17

TABLE 3. Composition of the Phenolic Components of Grape Cells Walls

Phenolic component*	Amount in the preparations, wt. %		Phenolic component*	Amount in the preparations, wt. %	
	$UCWs_0$	$DCWs_0$		$UCWs_0$	$DCWs_0$
Phenolic acids			Phenolic acids		
salicylic	0,42	—	syringic	0,12	—
cis-cinnamic	0,15	—	gallic	0,17	—
p-hydroxybenzoic	0,03	—	caffeic	0,11	0,03
vanillic + gentisic	Tr.	0,05	sinapic	0,06	0,01
protocatechuic + shikimic	0,02	0,11	chlorogenic	Tr.	0,09
			Sum	1,06	0,29

*(—)-Epicatechin, epigallocatechin, and quinic and ferulic acids were detected in trace amounts. Compounds with retention temperatures of 270-334°C were present in the preparations in amounts of between 1.3 and 1.7 wt.%.

100°C for 5 h). The sulfuric acid hydrolysates were neutralized with $BaCO_3$, and samples for chromatography were prepared on the basis of the results of the phenol-sulfuric acid reaction.

The protein component was hydrolyzed with 6 N HCl, and samples for chromatography were prepared by evaporation, dissolution in 0.1 N HCl, and sorption on Dowex 50 × 4 cation-exchange resin (H^+ form) with elution by 5% ammonia and reevaporation.

The alkaline hydrolysis of the bound substances was carried out in a current of nitrogen with 2 N NaOH (20°C, 5 h). The hydrolysates were neutralized and extracted three times with ethyl acetate, and the extract was evaporated to dryness. The preparation of sample before chromatography included standard silylation.

Chromatography of the monosaccharides was carried out on "Leningradskaya medlennaya [slow] No. 85" paper and on plates with activated cellulose (80°C, 1 h). The following solvent systems were used: ethyl acetate—acetic acid—water (4:1:3) (PC); and n-butanol—ethyl acetate—n-propanol—acetic acid—water (7:20:12:7:6) (TLC). Conditions of separation: descending chromatography, four runs, 50 h (PC); ascending chromatography, four runs, 24 h (TLC). The spots were revealed with a 2% ethanolic solution of p-aminobenzoic and phthalic acids (105°C, 10 min). The mixture for elution and quantitative analysis was 1.5% HCl in acetone (370 nm).

The ion-exchange chromatography of the amino acids was performed with the aid of a AAA-881 automatic analyzer (Czechoslovakia). Column 0.6 × 55 cm filled with KU-2 (H^+-form), two-step elution gradient at pH 3.25 and 4.25; rate of flow of buffer 70 ml/h; ninhydrin reagent, 35 ml/h.

The gas chromatography of the phenolic compounds was performed on a Chrom-5 chromatograph (Hungary). Glass column 250 × 0.2 cm; liquid phase 10% E-301 on Chromaton NAW-HMDS. Carrier gas helium at 30 ml/min; programming from 100 to 340°C (4°C/min).

CONCLUSIONS

1. The complex of high-molecular-weight substances of the cell walls of the grape has been studied.

2. It has been shown that the complex includes polysaccharides, proteins, and phenolic substances linked presumably through O-glycosidic bonds.

LITERATURE CITED

1. V. N Ezhov and E. N. Datunashvili, Prikl. Biokhim. Mikrobiol., 10, 908 (1974); V. I. Zinchenko, Polysaccharides of the Grape and of Wine [in Russian], Moscow (1978), p. 152; E. N. Datunashvili, Author's Abstract of Doctoral Dissertation, Moscow (1974).
2. E. N. Datunashvili, E. G. Manrikyan, and V. N. Ezhov, Biol. Zh. Arm., 7, 682 (1979).
3. T. Ahiyama, M. Mori, and K. Kato, Agric. Biol. Chemistry, 44, 2487 (1980); M. T. Esquerre-Tugaye and D. Masau, Physiol. Verg., 19, 415 (1981); G. S. Holst, F. M. Keis, and J. Buijs, Planta Physiol., 68, 910 (1981); M. M. Smit, R. D. Hartley, Biochem. Soc. Trans., 9, 165 (1981).
4. E. N. Datunashvili and V. N. Ezhov, in: Methods for Raising the Stability of Wines and Wine Materials [in Russian], Moscow (1982), p. 85.
5. H. Fausch, W. Kundig, and H. Neuhom, Nature (London), 193, 287 (1963); M. Knee, Phytochemistry, 12, 637 (1973); D. T. A. Lamport, Biochemistry, 8, 637 (1969).

IDENTIFICATION OF PROSTAGLANDINS E (SERIES 1 AND 2) ISOLATED FROM THE LIVING TISSUES OF *Larix sibirica* AND *Populus balzamifera*

É. D. Levin, Sh. T. Alaudinov, and V. É. Cherepanova

UDC 581.192.2:674.032.475.3

Prostaglandins E_1 and E_2 have been isolated for the first time from living tissues of the Siberian larch and the southern pine. The crude extract was purified by column and preparative thin-layer chromatography. The PGEs were identified by TLC and GLC, by UV and IR spectroscopy, and by chromato-mass spectrometry.

Since the time of the discovery of the prostaglandins (PGs), their universal presence in the organs and tissues of animals has been established [1]. The wide participation of the PGs in various biochemical processes that may take place not only in the higher animals but also in plants has permitted the assumption that the processes of the vital activity of the latter take place with the participation of PGs. This has served as a stimulus for the active search for PGs in representatives of the vegetable kingdom. However, until recently attempts to detect PGs in plants had proved unsuccessful. There are only isolated reports in the literature concerning the isolation of plant PGs, some of which are of a doubtful nature. K. A. Attrep et al. [2] isolated from the yellow onion (*Allium cepa*) a subtance "similar to or identical with PGA." The preparation was characterized with the aid of the methods of TLC and IR and UV spectroscopy. Later, with the aid of the TLC method, using ten solvent systems, in comparison with standard PGA_1, and also by the chromato-mass-spectrometric analysis of various derivatives and biotesting on the lowering of the blood pressure in comparison with standard PGA_1, the authors came to the definitive conclusion of the presence of PGA_1 in the onion [3]. R. P. Gregson et al. [4] were the first who succeeded in isolating PGE_2 and $PGF_{2\alpha}$ in the individual state from an aqueous extract of red algae (*Gracilaria lichnenoides*). The prostaglandins were identified with the aid of the methods of TLC, PMR, and ^{13}C NMR spectros-

Siberian Technological Institute, Krasnoyarsk.

Translated from Khimiya Prirodnykh Soedinenii, No. 5, pp. 567-571, September-October, 1984. Original article submitted by September 22, 1983.

0009-3130/84/2005-0533$08.50