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.

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

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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-

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TABLE 1. Values of m/z and Relative Intensities of the Ions (fragments) in the Mass Spectra of Derivatives of PGE_1 and PGE_2 of Plant Origin (%)

Ion	PGE_1		PGE_2	
	$(Me_3Si)_3-Me$	$(Me_3Si)_4$	$(Me_3Si)_3-Me$	$(Me_3Si)_4$
M	584(5)	642(2)	582(30)	640(10)
M—Me	569(6)	627(11)	567(8)	625(17)
M—$(Me_3Si)OH$	494(51)	552(100)	492(59)	550(70)
M—$2(Me_3Si)OH$	404(6)	462(75)	402(20)	460(100)
M—C_{16-20}	513(4)	571(3)	511(4)	569(5)
M—(C_{16-20})—$(Me_3Si)OH$	423(30)	481(50)	421(30)	479(40)
M—C_{15-20}	411(100)	469(79)	409(87)	467(39)
M—(C_{15-20})—$(Me_3Si)OH$	321(8)	379(42)	319(20)	377(49)
M—C_{13-20}	385(6)	443(6)	383(10)	441(16)
M—(C_{13-20})—$(Me_3Si)OH$	—	353(1)	293(10)	351(43)
M—(C_{1-5})—$2(Me_3Si)OH$	—	289(2)	287(6)	287(22)
M—C_{1-7}	—	441(3)	—	441(10)
M—(C_{1-7})—$(Me_3Si)OH$	351(10)	351(17)	—	351(13)
M—(C_{1-7})—$2(Me_3Si)OH$	261(4)	261(6)	261(10)	261(16)
C_{13-20}	199(5)	199(18)	199(11)	199(24)
C_{15-20}	173(27)	173(56)	173(100)	173(50)

copy, and mass spectrometry in comparison with standard PGE_2 and $PGF_{2\alpha}$ and with literature information. B. Janistin [5] detected $PGF_{2\alpha}$ in the flowering plant *Kalanchoe blossfeldiana* v. Poelln. The substance isolated was analyzed by TLC and chromato-mass spectrometry in comparison with standard $PGF_{2\alpha}$. There are also reports on the isolation from plants of substances containing a fragment of the carbon skeleton of the prostaglandins [6-8], which may be by-products of the metabolism of the PGs. G. S. Bild et al. [9] attempted to achieve the biosynthesis of PGs with the aid of plant enzymes for the first time. They incubated arachidonic acid with an isoenzyme of soybeans — lipoxygenase-2 — catalyzing the double peroxidation of arachidonic acid. As a result they obtained and identified several hydroxylated arachidonic acid derivatives and appreciable amounts of $PGF_{2\alpha}$. The latter compound could not reliably be assigned to the products of biosynthesis, since it was known from other sources [10] that under the conditions of biosynthesis used by these authors the formation of PGs from acid precursors can also take place partially even in the absence of enzymes. Information is given in the literature on the detection of prostaglandin-like compounds in plants. Thus, C. M. M. Cao et al. [11] isolated a fraction of prostaglandin-like compound "indistinguishable from PGE_2" from three species plants (*Saccharum officinarum*, *Musa paradisiaca*, and *Cocos Nucifera*). However, the methods of identification used by these authors (TLC, UV, and IR spectroscopy) are not sufficient to assign the preparations isolated to the PGs. A. G. Panosyan et al. [12] carried out a search for substances with a prostaglandin-like activity in a number of plants used in folk medicine as hypotensive, antiinflammatory, abortive, and antihemorrhagic agents. The chromatographic characteristics of the substances that they isolated were close to those of $PGF_{2\alpha}$, but their bioactivity was only 0.01-0.1% of the activity of $PGF_{2\alpha}$, and an analysis of them by chromato-mass spectrometry and PMR spectroscopy [13] showed that these authors were dealing with unsaturated C_{18} acids each containing three hydroxy groups.

We have undertaken a search for prostaglandins in the living tissues of trees. We previously reported a determination by a biological method of a prostaglandin-like action of compounds isolated from the cambial zone and buds of *Larix sibirica* [14]. In later communications, the characteristics of the total PGs isolated both from *Larix sibirica* and also from *Populis balzamifera* were described [15, 16]. Then the identification of $PGF_{1\alpha}$ was performed [17]. In the present paper we describe the identification of PGE_1 and PGE_2 isolated from the cambial zone and the buds of *Larix sibirica* and *Populis balzamifera* which are coniferous and broad-leaved species of higher plants. The identification was carried out by the methods of thin-layer chromatography (TLC), gas-liquid chromatography (GLC), UV and IR spectroscopy, and chromato-mass spectrometry.

For the preliminary purification of a crude extract of PGs and the isolation of fractions with a predominant content of type E PGs we used column chromatography. The process of separation was monitored by TLC on Silufol UV-254 plates in system 1 and also on glass plates coated with silica gel L in system 2 in comparison with standard $PGE_{2\alpha}$.* In each case, the R_f value of the preparation isolated coincided with that of the standard. It was 0.23-0.25 in system 1 and 0.57-0.60 in system 2.

After column chromatography, the fraction containing the PGEs was purified further by double preparative TLC in system 1. UV and IR spectra of the preparation obtained were recorded.

The UV spectrum of the sample after treatment with alkali showed an absorption maximum at 278 μ, which is characteristic for the prostaglandins of group E [18]. The IR spectrum of the preparation coincided completely with literature information characterizing the PGEs (ν, cm^{-1}): 3500-3200, 1715, 1705, 965 [19].

For gas-chromatographic analysis, the prostaglandins were converted into volatile derivatives: The carboxy groups were methylated with diazomethane, and the alcoholic hydroxyls were converted into trimethylsilyl ethers. The corresponding derivative of the standard PGE_2 was used as marker. On a chromatogram the retention times of the substance under investigation and of the standard coincided. A confirmation of the correctness of the identification was obtained by means of a mixed melting point.

By chromato-mass spectrometry using a glass capillary column, the PGE fraction was separated into two individual components. The retention times of the $(Me_3Si)_3$-Me derivatives of PGE_1 and PGE_2 were 6.50 and 6.25 min, and those of $(Me_3Si)_4$ derivatives 8.00 and 7.85 min, respectively.

The results of the mass-spectrometric analysis are given in Table 1. It can be seen from this table that the nature of the fragmentation and the sizes of the fragments formed are characteristic of PGE_1 and PGE_2 isolated from animal materials [20].

EXPERIMENTAL

The prostaglandins were isolated by methods similar to those given in the literature [21]. For column chromatography we used a 120 cm × 20 mm glass column. The adsorbent was 200 g of silica gel that had been calcined at 120°C for 16 h. The eluting mixtures were chloroform–ethanol with gradually increasing amounts of the latter. To accelerate the separation, chromatography was performed at an elevated pressure ($13 \cdot 10^4$ Pa).

The TLC of the extract was performed on Silufol UV 254 plates using the solvent system chloroform–methanol–acetic acid–water (90:8:1:0.8) (system 1) and on glass plates coated with silica gel L (5-40 μm) containing 5% of gypsum in the solvent system benzene–dioxane–acetic acid (20:20:1) (system 2). As the revealing agents were used a 10% ethanolic solution of molybdophosphoric acid and a 2% ethanolic solution of vanillin.

The UV spectra were obtained by using a SF-16 spectrophotometer. The range of wavelengths was from 230 to 300 μm. The sample was analyzed before and after its treatment with 2 N ethanolic potassium hydroxide.

The IR spectra of the methyl esters of the PGEs were recorded on a UR-20 instrument in chloroform. These methyl esters were obtained by treating the preparation with diazomethane. The spectra were recorded in the range of wavelengths of from 3500 to 700 cm^{-1} with the aid of a double-beam scheme with compensation for the absorption of the solvent.

The GLC analysis of the preparation was carried out with the aid of a Chrom-5 chromatograph (Czechoslovakia) with a flame-ionization detector. The separation was performed in a silanized glass column 2.5 m × 3 mm filled with Chromaton N-AW-DMCS (100-200 mesh) upon which 1% of the stationary phase SE-30 had been deposited. The column temperature was programmed from 180 to 280°C at a rate of heating of 5°C/min, and the carrier gas was helium at the rate of 35 ml/min.

*The standard PGE_2 was isolated from ram testes in the prostaglandin laboratory (director Prof. Yu. É. Lille) of the Institute of Chemistry of the Academy of Sciences of the Estonian SSR.

The chromato-mass spectrometric analysis of the extracts of prostaglandins from the living tissues of trees was carried out on a KLB-2109 chromato-mass spectrometer with substances in the form both of the methyltris(trimethylsilyl) derivatives $(Me_3Si)_3$-Me-PGE and in the form of the tetrakis(trimethylsilyl) derivatives $(Me_3Si)_4$-PGE.*

The preparation was methylated with freshly prepared solution of diazomethane, and was silylated by treating it with a mixture of N,O-bis(trimethylsilyl)trifluoroacetamide and piperidine (1:1) at 60°C for 1 h [22]. The reaction mixture was injected into the chromatograph directly.

Conditions of analysis: quartz capillary column 25 m × 0.35 mm; stationary phase SE-30; carrier gas helium, $P = 10.8 \cdot 10^4$ Pa; programming of the temperature from 200 to 300°C at a rate of heating of 10°C/min. The temperature of the separator 300°C and of the ion source 250°C; energy of the ionizing electrons 22.5 eV.

The mass spectra were recorded in the range of masses from 40 to 660 m/z.

CONCLUSION

The presence of prostaglandins E_1 and E_2 in the living tissues of the Siberian larch and the southern poplar have been shown for the first time by the methods of thin-layer and gas liquid chromatography UV, and IR spectroscopy, and mass spectrometry.

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*K. K. Pivnitskii, the director of the laboratory of prostaglandin and steroid chemistry of the Institute of Experimental Endocrinology and Hormone Chemistry, Academy of Medical Sciences of the USSR, made available to us the procedure and the recording of the mass spectra of the preparations.