

Polysaccharide from the Cherry-Tree Gum (*Prunus avium* L. var. *duracina* L.)

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Acidic polysaccharide containing D-glucuronic acid, 4-O-methyl-D-glucuronic acid, D-galactose, D-mannose, L-arabinose, and D-xylose has been isolated from the cherry-tree gum (*P. avium* L. var. *duracina* L.). Degraded polysaccharide was obtained in addition to L-arabinose, D-xylose, and D-galactose on hydrolysis. The polysaccharide was subjected to acid hydrolysis and products obtained were separated by paper chromatography and identified. Both original and degraded polysaccharide were oxidized with periodate. The degraded polysaccharide was methylated. Based on the obtained results, the main chain is believed to be composed of D-galactose residues: $\rightarrow 6\text{-}\beta\text{-D-Galp-1} \rightarrow 6\text{-}\beta\text{-D-Galp-1} \rightarrow$ to which D-glucuronic acid is attached at C-6 position. The side chains are formed by D-xylose, D-galactose, L-arabinose, and 4-O-methyl-D-glucuronic acid, respectively.

Structural features of polysaccharides from gums of two cherry-tree species have been reported in our previous studies. An acidic polysaccharide has been prepared from the wild cherry-tree gum (*P. avium* L. subsp. *avium*) [1] which afforded a homologous series of aldouronic acid $\beta\text{-D-GpA-1} \rightarrow [6\text{-}\beta\text{-D-Galp-1}]_{n=0-2} \rightarrow 6\text{-D-Gal}$ and 2-O- $[\beta\text{-D-GpA}]\text{-D-Man}$ on partial acid hydrolysis. A totally methylated degraded polysaccharide was also prepared and the hydrolytic products were determined by both paper and gas chromatography. The principal chain of this polysaccharide was formed by D-galactopyranose residues linked mostly by $\beta(1 \rightarrow 6)$ linkages. The side chains were composed of L-arabinose, D-xylose, and D-galactose. An acidic polysaccharide prepared from the cherry-tree gum (*P. avium* L. var. *juliana* L.) [2] gave a homologous series of aldouronic acids $\beta\text{-D-GpA-1} \rightarrow [6\text{-D-Galp-1}]_{n=0-3} \rightarrow 6\text{-D-Gal}$ as a main product on partial acid hydrolysis and was shown to have a basic chain of $\beta(1 \rightarrow 6)$ linked D-galactopyranose units. Some units in the polysaccharide were substituted in the C-6 position by D-glucuronic acid unit, in the C-3 position by a D-xylopyranose unit, and by L-arabinose units (maximum four), respectively.

The gum for the present investigation was collected on a *P. avium* L. var. *duracina* L. tree. Polysaccharide isolated in the known manner [1, 2] had an equivalent weight of 1790; $\bar{M}_{s,D} = 156\ 000$; $[\alpha]_D = -19^\circ$ ($c = 0,5$ in 1 M-NaOH); $-\text{OCH}_3 = 0,77\%$ and gave D-glucuronic acid, 4-O-methyl-D-glucuronic acid, D-galactose, D-mannose, L-arabinose, and D-xylose in a molar ratio of 5 5 30 5 72 7, and traces of L-rhamnose on total hydrolysis. The polysaccharide was homogeneous in ultracentrifuge.

The polysaccharide was subjected to a partial hydrolysis and the hydrolyzate was percolated through a column of Dowex 1 anion exchange resin to adsorb the acidic

* At temporary stay 1966—1967.

sugars. The neutral sugars were eluted with water and the following sugars were separated and identified by paper chromatography: D-mannose, L-rhamnose, D-xylose, L-arabinose, D-galactose, and three oligosaccharides based on D-galactose units being a homologous series of di-, tri- and tetrasaccharide, respectively (Fig. 1). We failed to identify the type of the linkage between D-galactose units due to the shortage of the material. Their chromatographic mobilities were as follows: $R_{\text{GalA}} = 0.27, 0.18, \text{ and } 0.06$. Acidic sugars were desorbed by gradient elution with acetic acid [3] and a homologous series of acidic oligosaccharides I–VI was obtained (Fig. 2)

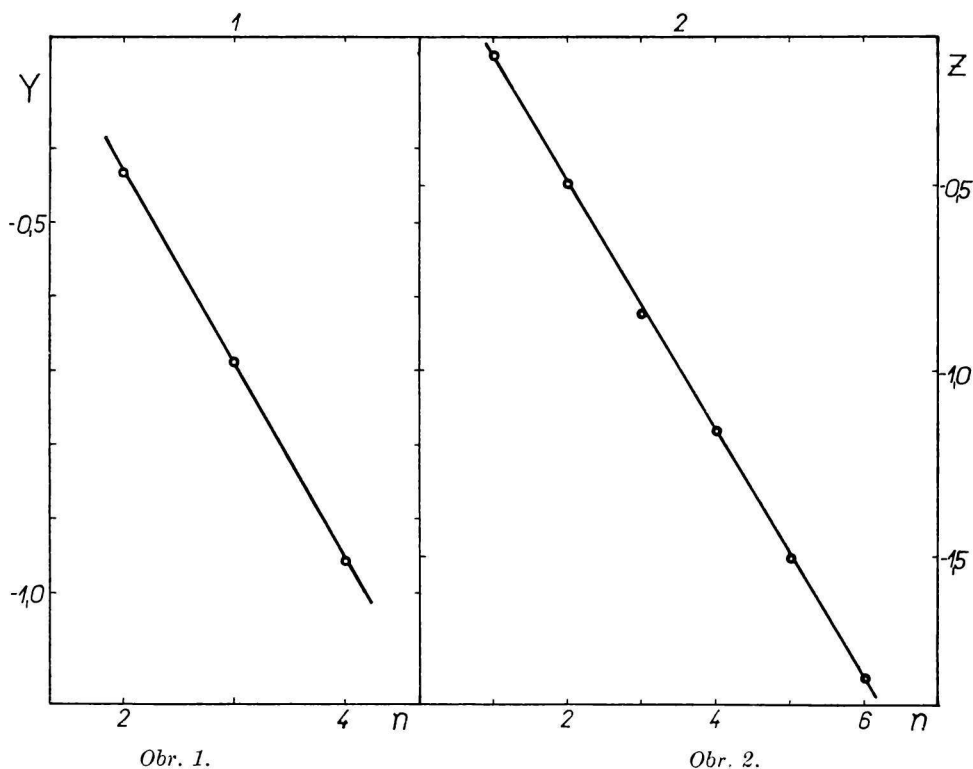


Fig. 1. Homologous series of the oligosaccharides based on D-galactose units.

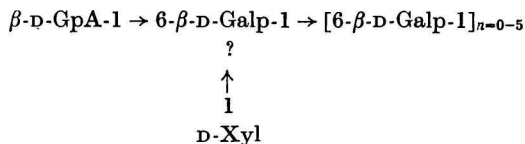
$$Y = \log \frac{R_{\text{Gal}}}{1 - R_{\text{Gal}}};$$

$n = \text{number of the D-galactose units.}$

Fig. 2. Homologous series of the oligosaccharides I–VI.

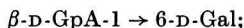
$$Z = \log \frac{R_{\text{GalA}}}{1 - R_{\text{GalA}}},$$

$n = \text{number of the D-galactose units in the oligosaccharides I–VI.}$

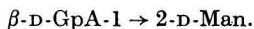


we failed to determine the position to which D-xylose units are attached;

oligosaccharide *VII*:



oligosaccharide *VIII*:



The oligosaccharides *I*—*VIII* were identified on the basis of partial hydrolysis and authentic samples.

Degraded polysaccharide with an equivalent weight of 950; $[\alpha]_{\text{D}} = +2^{\circ}$ ($c = 1$ in water); $\bar{M}_{\text{s,D}} = 40\,000$; $-\text{OCH}_3 = 1,22\%$ was obtained on autohydrolysis in a yield of 44% and was water-soluble. The degraded polysaccharide contained D-glucuronic acid, 4-*O*-methyl-D-glucuronic acid, D-galactose, D-mannose, L-arabinose, and D-xylose in a molar ratio of 5 : 5 : 27 : 5 : 13 : 6. As low molecular products, L-arabinose, D-xylose, and a small amount of D-galactose were split off on autohydrolysis.

Both original and degraded polysaccharide was oxidized by periodate. The periodate uptake and the amount of the resulting formic acid were 6.4 and 2.0 or 4.7 and 2.3, respectively, calculated on one equivalent of original and/or degraded polysaccharide. The oxidation product of the original polysaccharide was found to contain D-galactose and L-arabinose in the molar ratio of 11 : 10, and traces of D-xylose. 4-*O*-Methyl-D-glucuronic acid, D-mannose, and L-rhamnose were completely attacked by oxidation. The degraded polysaccharide contained D-galactose and L-arabinose in the molar ratio of 47 : 10 after the periodate oxidation. D-Glucuronic acid was identified in both oxidation products.

The degraded polysaccharide was methylated with dimethyl sulfate and sodium hydroxide. The partially methylated product was subsequently methylated with methyl iodide and silver oxide. One part of the methylated product was hydrolyzed with 72% aqueous sulfuric acid [4]. 2,3,4-Tri-*O*-methyl-D-xylose, 2,3,5-tri-*O*-methyl-L-arabinose, 3,4,6-tri-*O*-methyl-D-mannose, 2,3,4-tri-*O*-methyl-D-galactose, 2,3,4-tri-*O*-methyl-D-glucuronic acid, and an unidentified di-*O*-methyl-L-arabinose were identified by paper chromatography.

On the basis of the present evidence the following conclusion can be drawn. The principal chain is composed of D-galactose units: $\rightarrow 6\text{-}\beta\text{-D-Galp-1} \rightarrow 6\text{-}\beta\text{-D-Galp-1} \rightarrow$. To some of the D-galactose units, D-glucuronic acid is attached at C-6 position. The side chains are formed by D-xylose, D-galactose, L-arabinose, and 4-*O*-methyl-D-glucuronic acid.

Comparison of the previous results [1, 2] with the present ones shows that all polysaccharides are very similar each to other except that from the cherry-tree gum (*P. avium* L. var. *duracina* E.) which contains also 4-*O*-methyl-D-glucuronic acid.

Experimental results of the present and the earlier two studies [1, 2] are in Tab. 1.

Table 1
Physico-chemical constants of the polysaccharides from the cherry-tree gums

| Constants | Polysaccharides from gums (<i>Prunus avium</i> L.) | | | | | |
|------------------------------------------------------------------------|-----------------------------------------------------|--------------------------------------|------------------------------------------|-----------------------------------------|------------------------------|----------------------------|
| | subsp. <i>avium</i> | | <i>var. juliana</i> L. | | <i>var. duracina</i> L. | |
| | original | degraded | original | degraded | original | degraded |
| Equivalent weight | 1990 | 1190 | 1760 | 1030 | 1790 | 950 |
| Molar ratio 4MeGA : GA : Gal : Man : Ara : Xyl | $\emptyset : 10 : 45 : 1$ 70 : 10 | $\emptyset : 10 : 45 : 1$ 15 : 10 | $\emptyset : 10 : 32 : 5 :$: 70 : 10 | $\emptyset : 10 : 32 : 5 :$: 12 : 5 | 5 : 5 : 30 : 5 : : 72 : 7 | 5 : 5 : 27 : 5 : 13 : 6 |
| Periodate consumption (mole/equiv.) | 6.2 | 7.6 | 8.5 | 6.5 | 6.4 | 4.75 |
| Formation of HCOOH (mole/equiv.) | 3.0 | 2.4 | 3.25 | 3.5 | 2.35 | 2.65 |
| Molar ratio Gal : Ara : Xyl after oxidation with IO_4^- | 15 : 3 : 10 | 47 : 10 : \emptyset | 10 : 10 : \emptyset | 32 : 10 : \emptyset | 11 : 10 : \emptyset | 47 : 10 : \emptyset |
| Optical rotation $[\alpha]_D$ | +48° | +18° | +55° | +11° | -19° | +2° |
| Molecular weight $\overline{M}_{s,D} \cdot 10^{-3}$ | 431 | 43 | 250 | 38 | 156 | 40 |
| Sedimentation const. $s^{20} \cdot 10^{13}$ | 5.60 | 2.63 | 5.24 | 2.82 | 3.68 | 2.98 |

Experimental

Melting points were determined on a Kofler block. Optical rotations were measured at 20–22 °C.

Apparatus and procedures

Paper chromatograms were run on Whatman No. 1 and No. 3 papers by the descending method in the following solvent systems (v/v):

S₁: ethyl acetate—acetic acid—water 18 : 7 : 8;

S₂: ethyl acetate—pyridine—water 8 : 2 : 1;

S₃: *n*-butanol—ethanol—water 4 : 1 : 5.

Separated components were detected by the aniline hydrogen phthalate [5], 2,3,5-triphenyltetrazolium chloride [6], and alkaline silver nitrate [7] spray reagents. Chromatograms were quantitatively evaluated with Lange Chromatometer 3.

Equivalent weight was determined by potentiometric titration with 0.1 M-NaOH up to pH of 7.5 by automatic titrator TTTlc. Sedimentation constants were determined on an ultracentrifuge MOM G 110. Values of the diffusion coefficient and partial specific volume presented in [2] were used to calculate the molecular weight.

The periodate uptake and the formed formic acid produced were determined by the thiosulphate method [8] with amperometric indication [9]. Reducing sugars were determined by the phenol-sulfuric acid method [10].

Isolation of the polysaccharide

The gum was collected on one tree in Bratislava in August 1965. Crude gum (112 g) was dissolved in 2000 ml of water. Insoluble material was removed by filtration and centrifugation. The polysaccharide was prepared as described earlier [11]. Yield 76 g.

Hydrolysis of the polysaccharide

Quantitative hydrolysis was performed with 100 mg of both original and degraded polysaccharide in the known manner [12].

The original polysaccharide (10 g) was hydrolysed on a steam bath in 0.25 M-H₂SO₄ (750 ml) for 4 hours. The solution was passed through Zerolit 225 (H⁺ form) to adsorb Ba²⁺ ions after neutralization with BaCO₃. The solution was then percolated through Dowex 1 exchange resin in acetate form. Neutral saccharides (7.3 g) were eluted by water and preparative paper chromatography in the solvent system S₂ was then used for their separation: D-mannose and L-rhamnose identified only chromatographically; D-xylose (240 mg) chromatographically, $[\alpha]_D = +18^\circ$ (*c* = 1 in water), m. p. 143 °C; L-arabinose (353 mg), $[\alpha]_D = +103,8^\circ$ (*c* = 1 in water), m. p. 156 °C; D-galactose (207 mg), $[\alpha]_D = +76,1^\circ$ (*c* = 1 in water), m. p. 165 °C.

The acidic part was worked up [3] and five fractions were obtained. Fractions 3 (sirup, 54 mg, eluted with 4 M acetic acid), 4 (sirup, 191 mg, eluted with 4 M acetic acid), and 5 (sirup, 247 mg, eluted with 6 M acetic acid) moved slowly on chromatograms and were not investigated further.

In fraction 1 (sirup) 8 oligosaccharides were identified chromatographically and separated by preparative paper chromatography in solvent system S₁. D-Xylose was quantitatively split off during partial hydrolysis of the oligosaccharides I–VI with Zerolit 225

(H⁺ form) and a homologous series of oligosaccharides based on D-glucuronic acid and D-galactose was obtained. The oligosaccharides obtained were identified by paper chromatography using authentic samples [2] and on the basis of the linear dependence: $\log R_F/1 - R_F$ versus the number of members of the oligosaccharides [13]. Monosaccharides in these oligosaccharides are linked by β -glycosidic bonds as it is clear from the $[\alpha]_D$ values. Yields and physico-chemical constants of the oligosaccharides I–VI are in Tab. 2.

Table 2

Yields, chromatographic mobilities, and optical rotations of the oligosaccharides I–VI

| Oligosaccharide | Yield mg | R_{GalA} | $[\alpha]_D$ | Products of hydrolysis* |
|-----------------|----------|------------|--------------|-------------------------|
| I | 102.3 | 0.48 | −6,8° | Xyl; GA, Gal, 1 |
| II | 70.0 | 0.24 | −6,2° | Xyl; GA, Gal, 1, 2 |
| III | 21.4 | 0.12 | −1,6° | Xyl; GA, Gal, 1-3 |
| IV | 22.6 | 0.06 | −1,0° | Xyl; GA, Gal, 1-4 |
| V | 8.0 | 0.03 | −0,6° | Xyl; GA, Gal, 1-5 |
| VI | 3.1 | 0.02 | 0,0° | Xyl; GA, Gal, 1-6 |

* 1–6: β -D-GpA-1 \rightarrow [6- β -D-Galp-1] $\xrightarrow[n=0-5]{\text{---}}$ 6-D-Gal.

Oligosaccharides VII and VIII were identified after hydrolysis by paper chromatography only using authentic samples [1, 2].

Preparation of the degraded polysaccharide

The original polysaccharide (10 g) was suspended in 1300 ml of distilled water and the suspension heated on the steam bath. The course of autohydrolysis of the glycosidic linkages was checked by 3,5-dinitrosalicic acid [14]. The hydrolysis was completed after 32 hours. The solution was concentrated to 500 ml and poured in ethanol (1500 ml containing 1 % of hydrochloric acid) to precipitate the degraded polysaccharide. Yield 4.4 g. The filtrate was neutralized with silver carbonate and filtered again. The Ag⁺ ions were removed by Zerolit 225 in H⁺ form and the solution was concentrated in vacuo. D-Xylose, L-arabinose, traces of D-galactose, and oligosaccharides based on L-arabinose units were identified by paper chromatography in solvent system S₂.

Periodate oxidation

Both original and degraded polysaccharide (1 g) were oxidized analytically with 250 ml of 0.04 M sodium periodate 10 days at +2 °C in the dark. Further procedures as described earlier [2, 11].

Methylation of the degraded polysaccharide

The degraded polysaccharide (1.8 g) was methylated with dimethyl sulfate and sodium hydroxide in the known manner [15]. Partially methylated product was further methylated with methyl iodide and silver oxide [16]. The resulting product (1.58 g) of $[\alpha]_D =$

= -22,6° ($c = 1,2$ in chloroform), $-\text{OCH}_3 = 39,4$ -showed no OH absorption in the infra % red spectrum. One part of the methylated degraded polysaccharide was hydrolysed by 72 % sulfuric acid [4]. The methyl derivatives present in hydrolyzate were determined by paper chromatography in solvent systems S_2 and S_3 (authentic samples were chromatographed in the same run) (Tab. 3).

Table 3
Identification of the methylated saccharides

| R_G | Structure* |
|-------|-------------------------------------------------|
| 0.98 | 2,3,4-tri- <i>O</i> -methyl-D-xylose |
| 0.96 | 2,3,5-tri- <i>O</i> -methyl-L-arabinose |
| 0.89 | 3,4,6-tri- <i>O</i> -methyl-D-mannose |
| 0.80 | 2,3- (or 3,5-) di- <i>O</i> -methyl-L-arabinose |
| 0.76 | 2,3,4-tri- <i>O</i> -methyl-D-galactose |
| 0.58 | 2,4-di- <i>O</i> -methyl-D-galactose |
| 0.32 | 2,3,4-tri- <i>O</i> -methyl-D-glucuronic acid |

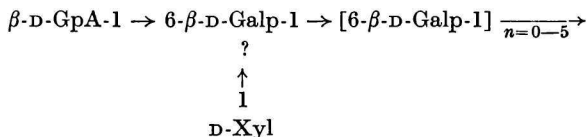
* Methyl derivatives of sugars were identified by paper chromatography using authentic samples.

POLYSACHARID Z GUMY ČEREŠNE (*Prunus avium* L. var. *duracina* L.)

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Z gummy čerešne (*P. avium* L. var. *duracina* L.) sa pripravil kyslý polysacharid o ekvivalentovej váhe 1790; molekulovej váhe $\overline{M}_{s,D} = 156\ 000$; $[\alpha]_D = -19^\circ$ ($c = 0,5$ v 1 M-NaOH); $-\text{OCH}_3 = 0,77\ %$. Obsahuje kyselinu D-glukurónovú, kyselinu 4-*O*-metyl-D-glukurónovú, D-galaktózu, D-manózu, L-arabínózu, D-xylózu v molárnom pomere 5 : 5 : 30 : 5 : 72 : 7 a L-ramnózu v stopách. Autohydrolyzou sa získal degradovaný polysacharid o ekvivalentovej váhe 950, molekulovej váhe $\overline{M}_{s,D} = 40\ 000$; $[\alpha]_D = +2^\circ$ ($c = 1$ vo vode); $-\text{OCH}_3 = 1,22\ %$. Tento obsahuje kyselinu D-glukurónovú, kyselinu 4-*O*-metyl-D-glukurónovú, D-galaktózu, D-manózu, L-arabínózu a D-xylózu v molárnom pomere 5 : 5 : 27 : 5 : 13 : 6. Pôvodný polysacharid sa parciálne hydrolyzoval a získal sa homologický rad aldourónových kyselín:



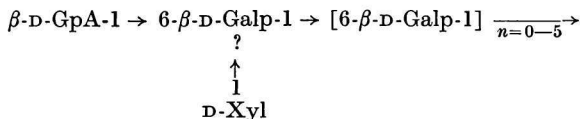
a aldobiurónové kyseliny: β -D-GpA-1 \rightarrow 6-D-Gal a β -D-GpA-1 \rightarrow 2-D-Man. Pôvodný aj degradovaný polysacharid sa oxidoval jodistanom. Degradovaný polysacharid sa metyloval a stanovili sa produkty hydrolyzy metylovaného degradovaného polysacharidu. Na základe získaných experimentálnych výsledkov sa predpokladá, že v hlavnom retazci sú jednotky D-galaktózy zoradené podľa vzorca: \rightarrow 6- β -D-Galp-1 \rightarrow 6- β -D-Galp-1 \rightarrow , ktorý je v polohách C-6 vetvený jednotkami kyseliny D-glukurónovej. Bočné retazce tvoria jednotky D-xylózy, D-galaktózy, ale najmä jednotky L-arabínózy. Štrukturálne črty tohto polysacharidu sa koreľujú so štrukturálnymi črtami príbuzných polysacharidov z gummy čerešne (*P. avium* L. var. *juliana* L.) a z gummy divej čerešne (*P. avium* L. subsp. *avium*).

ПОЛИСАХАРИД ИЗ КАМЕДИ ЧЕРЕШНИ (*Prunus avium* L. var. *duracina* L.)

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Из камеди черешни (*P. avium* L. var. *duracina* L.) был изолирован кислый полисахарид эквивалентного веса 1790; молекулярного веса $\bar{M}_{s,D} = 156\ 000$; $[\alpha]_D = -19^\circ$ ($c = 0,5$ в 1-м NaOH); $-\text{OCH}_3 = 0,77\%$, который содержит D-глюкуроновую кислоту, 4-O-метил-D-глюкуроновую кислоту, D-галактозу, D-маннозу, L-арабинозу, D-ксилозу в молекулярном соотношении 5 5 30 5 72 7 и L-рамнозу в следах. Автогидролизом был получен расщепленный полисахарид эквивалентного веса 950; молекулярного веса $\bar{M}_{s,D} = 40\ 000$; $[\alpha]_D = +2^\circ$ ($c = 1$ в воде); $-\text{OCH}_3 = 1,22\%$, который содержит D-глюкуроновую кислоту, 4-O-метил-D-глюкуроновую кислоту, D-галактозу, D-маннозу, L-арабинозу и D-ксилозу в молекулярном соотношении 5 5 27 5 13 6. Частичным гидролизом исходного полисахарида был получен гомологический ряд альдоурановых кислот:



и альдобиурановые кислоты: β -D-GpA-1 \rightarrow 6-D-Gal и β -D-GpA-1 \rightarrow 2-D-Man. Исходный а также расщепленный полисахарид окислялся периодатом натрия. Расщепленный полисахарид был метилирован и определились продукты гидролиза метилированного расщепленного полисахарида. На основании полученных экспериментальных данных предполагается, что в главной цепи находятся единицы D-галактозы сопоставленные по образцу: \rightarrow 6- β -D-Galp-1 \rightarrow 6- β -D-Galp-1 \rightarrow , который разветвленный в положениях C-6 единицами D-глюкуроновой кислоты. Боковые цепи образуют единицы D-ксилозы, D-галактозы, но особенно единицы L-арабинозы. Структурные черты этого полисахарида сопоставляются со структурными чертами родственных полисахаридов из камеди черешни (*P. avium* L. var. *juliana* L.) и из камеди дикой черешни (*P. avium* L. subsp. *avium*).

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Received July 7th, 1967

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