INFLUENCE OF CHLOROCHOLINE CHLORIDE AND OF TRACE ELEMENTS ON THE ACCUMULATION OF POLYSACCHARIDES IN *Solidago virgaurea*

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Continuing an investigation of the water-soluble polysaccharides (WSPs) of *Solidago virgaurea* L. (European goldenrod) [1, 2], we have studied the accumulation and composition of the WSPs under the influence of a 0.3% solution of chlorocholine chloride (the preparation TUR) and of a mixture of 0.05% solutions of trace elements (TEs): boric acid (H_3BO_3), the salts $CuSO_4 \cdot 5H_2O$, $ZnSO_4 \cdot 7H_2O$, $(NH_4)_2MoO_4$, and $MgSO_4 \cdot 7H_2O$, and a mixture of the latter with the retardant in a ratio of 1:1.

The plants were treated in 1983 in the morning hours twice; the first treatment before flowering (the budding phase) and the second treatment 10-12 days after the first. The consumption of the solutions was 40-50 ml per plant. As control we used plants treated with pure water. The raw material was gathered in the period of mass flowering.

The WSPs were extracted from the air-dry raw material with water and then they were demineralized and their ash contents were determined by published procedures [1, 3] and their uronic anhydride contents by potentiometric titration. The ash contents of the demineralized polysaccharides (PSs) were 0.8-1.0%. The hydrolysis of the PSs and the subsequent operations with them were performed as described previously [4]. The hydrolysates were studied by the PC method in the butan-1-ol—pyridine—water (6:4:3) system. The neutral sugars were revealed with aniline phthalate.

It was established that the qualitative monosaccharide composition (D-galacturonic acid, D-galactose, D-glucose, L-arabinose, D-xylose, L-rhamnose, and two unidentified monosaccharides) did not change under the influence of chlorocholine chloride and the MEs. The amounts of neutral monosaccharides present were determined by the procedure described in [4].

Variant of the experiment	Yield of the two SPs %	Ash content, %	Amounts of monosaccharides on the total taken as 100%					*Gal UA*, %
			Gal	*Glc*	*Ara*	*Xyl*	*Rha*	
Control	4,8	25 4	31,07	20 63	29,50	9,79	9,01	44,5
TUR	9,0	27,1	28,88	21,72	33,38	5,27	10,41	47,0
MEs	5,8	26,5	35,26	14,89	35,54	5,15	9,15	45,3
TUR + MEs	6 2	26,8	33,84	19,56	32,94	6,15	7,51	46,1

As we see, the SPs were distributed nonuniformly: The epigeal parts of the experimental plants contained a larger amount than in the control plants. The retardant increased the accumulation of WSPs 1.9-fold, its mixture with MEs did so 1.3-fold, and the MEs 1.2-fold. The samples of WSPs in the experiments with chlorocholine chloride and its mixture with the MEs were distinguished by a somewhat larger ($P < 0.001$) content of galacturonic acid than the control sample. Its accumulation in the PSs of the plants treated with the MEs was not significant ($P > 0.05$). No appreciable differences were detected in the amounts of ash in the WSPSs. The main components in the sugars and PSs were galactose, arabinose, and glucose. The chlorocholine chloride and the MEs acted on their biosynthesis differently. Thus, the retardant and a mixture of it with MEs had no influence ($P > 0.05$) on their amount in the WSPs, while the MEs, without changing the arabinose content, increased the level of galactose and lowered the amount of glucose. In all the variants of the experiment, the percentage amount of xylose fell considerably (by a factor of 1.5-1.9) and the amount of rhamnose remained at the level of the control.

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Thus, the increase in the yield of WSPs when European goldenrod plants were treated with chlorocholine chloride took place at the expense of the fraction of pectin substances. The MEs increased the yield of PSs somewhat more feebly, their increase taking place at the expense of the galactose. The combined treatment of the plants with both preparations showed an intermediate effect on the yield of WSPs. Their accumulation took place through an increase in the proportion of pectic substances, as in the experiments with the retardant.

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THE POSSIBILITY OF DETECTING BACTERIAL ENDOTOXINS BY THIN-LAYER CHROMATOGRAPHY

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In their chemical structure, bacterial endotoxins (or pyrogenic substances) are intricate complexes of lipopolysaccharides, phospholipids, and proteins of high molecular weight. At the present time, it is considered established that endotoxins are lipopolysacchrides [1]. The physicochemical properties of bacterial endotoxins are due, on the one hand, to a hydrophobic lipid A, and on the other hand, to highly polar polysaccharide radicals, and therefore the effective separation of such compounds requires polar mobile phases. It has been reported that the desorption of pyrogens from various agents sorbing them takes place most effectively with a phosphate buffer solution having pH 9-11 [2]. In the course of an experiment, the literature information has been confirmed completely, but the clearest round zones were obtained for a mobile phase with a pH of 11.05.

To detect bacterial endotoxins on chromatograms, the use of such reagents as Rhodamine 6G, molybdophosphoric acid, and iodine vapor [3], and Bromocresol Green [4] has been described. On comparing the sensitivity of detection of bacterial endotoxins by the reagents mentioned, it was established that the most sensitive was a 1% solution of molybdophosphoric acid (0.4 μg), and then a 0.1% solution of Bromocresol Green (0.8 μg), a 0.02% solution of Rhodamine 6G, and a 0.2% solution of fluorescein (1 μg). On this basis, as the detecting reagent we selected a 1% solution of molybdophosphoric acid.

We have investigated the lipopolysaccharides secreted by the typhus bacterium *Salmonella typhi* and used in medicine in the form of solutions with different biological activities under the name of Pyrogenal. As the sorbent we used Silufol prepared plates (Czechoslovakia).

Procedure for Detecting Pyrogenal. With the aid of a MSh-1 microsyringe, 0.3 μl of a solution of the polysaccharide (Pyrogenal) was deposited at the starting line of a Silufol plate with dimensions of 6 × 4 cm. After being dried with a stream of hot air, the plate was placed in a chamber with the mobile phase — a phosphate buffer solution having pH 11.05. After the solvent front had run a distance of 5 cm, the plate was taken out and dried in the air. Then the chromatogram so obtained was treated with a 1% solution of molybdophosphoric acid and it was kept at 160-170°C for 1-2 min. Grey zones of the polysaccharide with a R_f value of 0.75-0.78 appeared against a light yellow background. The sensitivity of detection was 0.4 μg of substance in the zone.

Thus, the basic possibility has been shown of using thin-layer chromatography for detecting bacterial endotoxins.

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