Distribution pattern of 4-*O*-methyl-D-glucuronic acid units in 4-*O*-methyl-D-glucurono-D-xylan isolated from the leaves of marsh mallow (*Althaea officinalis* L., var. *Rhobusta*)

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Dedicated to Professor P. Kristian, DrSc., in honour of his 60th birthday

A homogeneous, water-soluble 4-*O*-methyl-D-glucurono-D-xylan has been isolated from the leaves of marsh mallow. The mole ratio of D-xylose and 4-*O*-methyl-D-glucuronic acid was 7 : 1. The main chain was built of (1→4)-linked β-D-xylopyranosyl residues and 4-*O*-methyl-D-glucuronic acid was attached as single terminal units to O-2 of D-xylose.

The single-ion activity coefficient of calcium counterions $\gamma_{Ca^{2+}}$ was estimated in a molecular disperse solution of the polysaccharide. The mean distance of adjacent carboxyl groups, $b = 4.00$ nm, was obtained from experimentally determined value of $\gamma_{Ca^{2+}} = 0.712$, using the relation $\gamma_{Ca^{2+}} = f(b)$. From comparison of the b value with the length of the D-xylose unit (0.52 nm) it follows that the uronic acid units are apart and distributed regularly along the macromolecule chain, *i.e.* approximately each seventh D-xylose unit is substituted by 4-*O*-methyl-D-glucuronic acid.

Из листьев алтея аптечного выделен гомогенный водорастворимый 4-*O*-метил-D-глюкуроно-D-ксилан. Мольное отношение D-ксилозы и 4-*O*-метил-D-глюкуроновой кислоты в нем было 7 : 1. Главная цепь состояла из (1→4)-связанных остатков β-D-ксилопиранозы, причем 4-*O*-метил-D-глюкуроновая кислота была присоединена в виде одиночных концевых групп к атому O-2 D-ксилозы.

Величина одноионного коэффициента активности кальциевых противоионов $\gamma_{Ca^{2+}}$ была определена в молекулярно дисперсном растворе полисахарида. Значение среднего расстояния между соседними карбоксильными группами, $b = 4{,}00$ нм, было получено на основе опытно установленного значения $\gamma_{Ca^{2+}} = 0{,}712$, используя соотношение $\gamma_{Ca^{2+}} = f(b)$. Из сравнения величины b с размером единицы D-ксилозы (0,52 нм) следует, что единицы уроновой кислоты не находятся рядом, а регулярно распределены вдоль цепи макромолекулы, т.е. приблизительно каждая седьмая единица D-ксилозы замещена 4-*O*-метил-D-глюкуроновой кислотой.

Elucidation of sequential arrangement of 4-*O*-methyl-D-glucuronic acid in the D-xylan molecule is not an easy task. Partial acid or enzyme hydrolysis

followed by fractionation and characterization of oligomeric fragments is time-consuming and may lead to ambiguous conclusions. Earlier [1] it was generally accepted that in glucuronoxylans the monomeric uronic acid units are distributed randomly along the D-xylan chain. However, *Shimizu* and coworkers [2] isolated a tetramer containing two vicinal units of 4-*O*-methyl-D-glucuronic acid bound to O-2 of D-xylobiose from the hydrolyzates of hemicelluloses of neutral sulfite waste liquor of larch. Lately, *Kohn et al.* [3, 4] have suggested a block-wise distribution of 4-*O*-methyl-D-glucuronic acid in 4-*O*-methyl-D-glucurono-D-xylans isolated from the bark of white willow and beech. They came to this conclusion on the basis of the results obtained by a novel methodic approach, *i.e.* by determination of the single-ion activity coefficient of calcium counterions ($\gamma_{Ca^{2+}}$) in solutions of calcium salts of these polysaccharides.

In the present work we made use of this method in determination of distribution pattern of side 4-*O*-methyl-D-glucuronic acid units in 4-*O*-methyl-D-glucurono-D-xylan isolated from the leaves of marsh mallow (*Althaea officinalis* L., var. *Rhobusta*).

Experimental

Potentiometric titrations were performed on a PHM 64 (Radiometer, Copenhagen) potentiometer using a GK 2401 C combined electrode.

Spectrophotometric measurements were carried out on a Specol 11 (Zeiss, Jena) spectrometer.

The Cd spectra of both potassium and calcium salts of the polysaccharide were measured in 1.00 mmol dm^{-3} solutions of COOK and $COOCa_{0.5}$ with a Jobin Yvon Mark III (France) dichrograph.

Reagents used: 0.058 M-KOH, 0.021 M-$Ca(OH)_2$, 0.005 M-$CaCl_2$, redistilled water freed from carbon dioxide, and tetramethylmurexide synthesized according to the method described in [5].

Isolation and characterization of the polysaccharide

The isolation of 4-*O*-methyl-D-glucurono-D-xylan from the leaves of marsh mallow and its subsequent separation from the accompanying polysaccharides were carried out as described in our previous work [6]. The structural features, determined by methylation analysis and ^{13}C NMR spectroscopy, are described in the same work. The content of 4-*O*-methyl-D-glucuronic acid was determined by potentiometric titration and the methoxyl content according to the method of *Viebock* and *Brecher* [7]. Both methods afforded consistent results. The number average molecular mass ($\bar{M}_n$) was determined osmometrically at 30 °C after equilibration with 0.1 M-NaCl, using a Knauer membrane osmometer fitted with a Zweischicht-Membrane (Knauer).

Determination of activity of Ca^{2+} ions in the solution of calcium salt of 4-O-methyl-D-glucurono-D-xylan

The polysaccharide was converted into H^+ form by percolating the polysaccharide solution through Amberlite IR 120 (H^+) and then it was neutralized to the point of equivalence with saturated $Ca(OH)_2$ solution.

The activity of Ca^{2+} ions was determined in the solution of calcium salt of the polysaccharide ($c(COOCa_{0.5}) = 3.00\,mmol\,dm^{-3}$) by the spectrophotometric method using tetramethylmurexide ($c = 4 \times 10^{-5}\,mol\,dm^{-3}$) as the metallochromic indicator [8, 9]; the solution did not contain any additional inert electrolyte. The calibration curve was constructed from the data obtained with $CaCl_2$ solution. The single-ion activity coefficient $\gamma_{Ca^{2+}}$ was calculated from the Ca^{2+} activity and the total concentration of Ca^{2+} in the solution ($1.5\,mmol\,dm^{-3}$).

Determination of distribution of 4-O-methyl-D-glucuronic acid in the polysaccharide

The mean distance (b/nm) between two adjacent free carboxyl groups in their perpendicular projection on the axis of the D-xylan linear chain was estimated from the analytical curve $\gamma_{Ca^{2+}} = f(b)$. This function was introduced in our earlier paper [10] using $\gamma_{Ca^{2+}}$ determined in model solutions of variously esterified pectins ($E > 43\,\%$) [11].

Results and discussion

A homogeneous, water-soluble 4-*O*-methyl-D-glucurono-D-xylan has been isolated from the leaves of marsh mallow. The polysaccharide was composed of (1→4)-linked β-D-xylopyranosyl residues, ≈70 % being unsubstituted, ≈5 % carrying a single substitution at O-2 and/or O-3, and ≈11 % being doubly branched on O-2 and O-3. 4-*O*-Methyl-D-glucuronic acid was attached to O-2 of D-xylose as single terminal units. The mole ratio of D-xylose to 4-*O*-methyl-D-glucuronic acid in the polysaccharide was 7 : 1 (Table 1).

Table 1

Characterization of 4-*O*-methyl-D-glucurono-D-xylan

w(D-Xylose)	83.10 %
w(4-*O*-Methyl-D-glucuronic acid)	16.90 %
$w(OCH_3)$	2.72 %
$\bar{M}_n$	21 563
$[\alpha]$(D, 22 °C, $\varrho = 5.0\,g\,dm^{-3}$, water)	−77.3°
Mole ratio D-xylose : 4-*O*-methyl-D-glucuronic acid	7.09 : 1.00

The principle of the method for determination of the distribution pattern of carboxyl groups in an acid polysaccharide molecule, based upon interpretation of single-ion activity coefficient of calcium counterions ($\gamma_{Ca^{2+}}$), has already been described [10]. The prerequisite for applying the foregoing method is the electrostatic bond between the calcium ions and carboxyl groups of the polysaccharide. The activity coefficient of counterions (γ_i), bound by electrostatic bond to the polysaccharide, is a function of linear-charge density of the macromolecule characterized by the mean distance between two adjacent carboxyl groups (b/nm). The function $\gamma_{Ca^{2+}} = f(b)$ (Fig. 1) has a general validity for linear acid

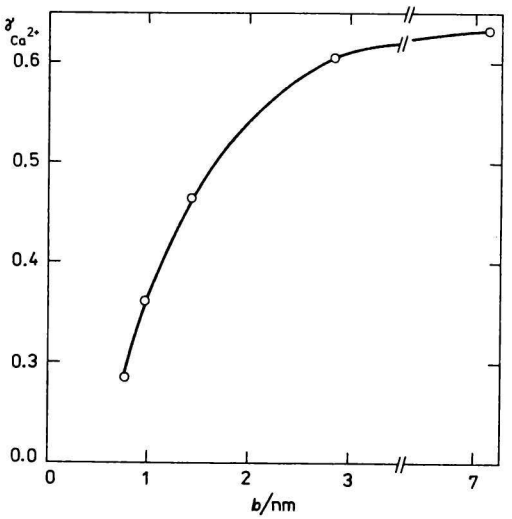

Fig. 1. Dependence of $\gamma_{Ca^{2+}}$ on the mean distance (b) of two vicinal free carboxyl groups in a linear polysaccharide.

polysaccharides, regardless of the kind of uronic acid units in the polysaccharide. As it was mentioned in Experimental, Fig. 1 was constructed from the values of activity coefficients determined in solutions of calcium salts of pectins having more than 43 % carboxyl groups esterified, wherein the bond between calcium and carboxyl groups was exclusively electrostatic [11].

The identity of circular dichroic spectra (Fig. 2) of calcium and potassium salts of the glucuronoxylan studied herein indicated that the bond between calcium and carboxyl groups was electrostatic [12]. The spectra, recorded with transparent solutions (concentration of COOK and $COOCa_{0.5} = 1.00\,mmol\,dm^{-3}$), revealed the maximum in the negative value of the ellipticity range at $\lambda = 210\,nm$.

The activity of Ca^{2+} counterions ($a_{Ca^{2+}}$) was estimated in the solution of calcium salt of 4-*O*-methyl-D-glucurono-D-xylan ($c(COOCa_{0.5}) = 3\,mmol\,dm^{-3}$) and the activity coefficient $\gamma_{Ca^{2+}} = 0.712$ was calculated. This value is very

similar to that found at the same concentration with calcium D-glucuronate ($\gamma_{Ca^{2+}} = 0.755$).

The distance between two vicinal carboxyl groups in the polysaccharide

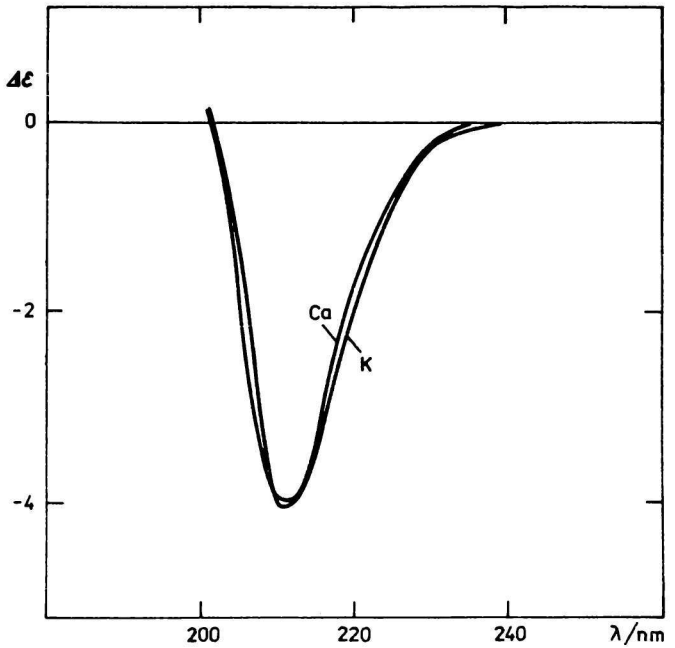

Fig. 2. Circular dichroic spectra of potassium and calcium salts of 4-*O*-methyl-D-glucurono-D-xylan (concentration of COOK and $COOCa_{0.5}$ = 1 mmol dm^{-3}).

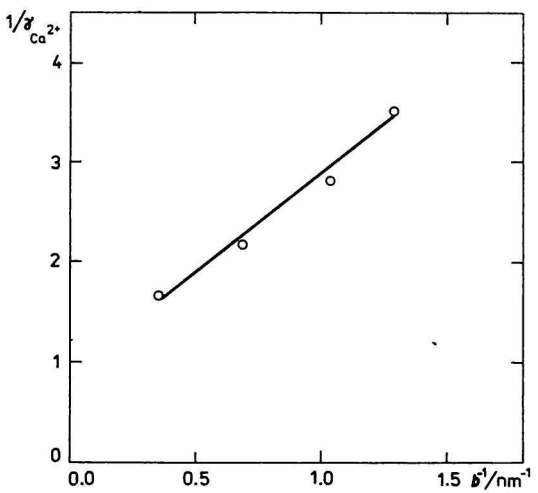

Fig. 3. Linearized form ($1/\gamma_{Ca^{2+}} = f(1/b)$) of the relationship illustrated in Fig. 1.

$b = 4.00$ nm, corresponding to the found activity coefficient, was obtained from the linearized form ($1/\gamma_{Ca^{2+}} = f(1/b)$; Fig. 3) of the function $\gamma_{Ca^{2+}} = f(b)$ (Fig. 1). Though this relationship was proved to be valid for linear acid polysaccharides [11], *i.e.* when the uronic acid units are built in the linear chain, we consider this dependence applicable to our polysaccharide, since 4-*O*-methyl-D-glucuronic acid is attached to the main linear chain exclusively as single terminal units.

When considering the analogy with the macromolecule of D-mannuronan, having the same type of glycosidic bonds (β-(1→4)) of the saccharide units in $^4C_1$ conformation, the length of one D-xylose unit in the D-xylan chain is 0.52 nm [13]. Then from the b value 4.00 nm it follows that approximately each seventh D-xylose unit in the macromolecule is substituted by one unit of 4-*O*-methyl-D--glucuronic acid. This conclusion is in agreement with the results of structural studies.

Table 2

Mean distance between two adjacent carboxyl groups (b) in 4-*O*-methyl-D-glucurono-D-xylans in a perpendicular projection on the main axis of their molecules

Source	Mole ratio Xyl : UA	b/nm
Bark of white willow [3]	6.0	1.10
Beech wood [4]	6.1	1.20
Leaves of marsh mallow	7.0	4.00

For comparison, we present in Table 2 the b values determined with 4-*O*--methyl-D-glucurono-D-xylans isolated from the bark of white willow and beech wood [3, 4]. These point to irregular distribution of uronic acid units in the macromolecule. The polysaccharides are composed of blocks with more frequent branching (approximately at each second xylose unit), alternating with those composed exclusively of xylose units or containing only small amount of uronic acid units. Contrary to these polysaccharides, in the 4-*O*-methyl-D--glucurono-D-xylan studied herein, the uronic acid units are apart and distributed regularly along the macromolecule chain. This finding completes the knowledge on the structure of this polysaccharide. Moreover, it may be of importance in view of investigations and possible classifications of further types of glucuronoxylans or hemicelluloses in general, considering their origin.

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References

1. Timell, T. E., *Adv. Carbohydr. Chem. 19*, 247 (1964).
2. Shimizu, K. and Samuelson, O., *Svensk Papperstidn. 76*, 156 (1973).
3. Toman, R., Kohn, R., Malovíková, A., and Rosík, J., *Collect. Czechoslov. Chem. Commun. 46*, 1405 (1981).
4. Kohn, R., Hromádková, Z., Ebringerová, A., and Toman, R., *Collect. Czechoslov. Chem. Commun. 51*, 2243 (1986).
5. Gysling, H. and Schwarzenbach, G., *Helv. Chim. Acta 32*, 1484 (1949).
6. Kardošová, A., Capek, P., and Rosík, J., *Chem. Papers 43*, 705 (1989).
7. Viebock, F. and Brecher, C., *Ber. 63B*, 3207 (1930).
8. Kohn, R. and Furda, I., *Collect. Czechoslov. Chem. Commun. 32*, 1925 (1967).
9. Kohn, R., *Chem. Zvesti 28*, 625 (1974).
10. Kohn, R., Rosík, J., Kubala, J., and Malovíková, A., *Collect. Czechoslov. Chem. Commun. 44*, 2517 (1979).
11. Kohn, R. and Luknár, O., *Collect. Czechoslov. Chem. Commun. 40*, 959 (1975).
12. Kohn, R. and Sticzay, T., *Collect. Czechoslov. Chem. Commun. 42*, 2372 (1977).
13. Atkins, E. D. T., *Pure Appl. Chem. 49*, 1135 (1977).

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