Polymer Bulletin 60, 775–783 (2008)
DOI 10.1007/s00289-008-0918-6

Polymer Bulletin

Unconventional Synthesis of Pullulan Abietates

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Received: 19 December 2007 / Revised version: 13 February 2008 / Accepted: 26 February 2008
Published online: 10 March 2008 –

Summary

Pullulan abietic acid esters (pullulan abietates) of different degree of substitution (DS) were synthesized homogeneously in *N,N*-dimethylacetamide using differently *in situ* activated abietic acid derivatives. *In situ* activation was achieved with *p*-toluenesulfonyl chloride, *N,N`*-carbonyldiimidazole and iminium chloride formed from oxalyl chloride/*N,N*-dimethylformamide. The DS values of the biopolymer esters determined by acid-base titration after saponification indicated that *in situ* activation with *p*-toluenesulfonyl chloride is most efficient while in case of the *in situ* activation with *N,N`*-carbonyldiimidazole almost no polymer degradation occured. The pullulan abietates were characterized by elemental analysis, GPC, FTIR-, ^{1}H- and ^{13}C NMR spectroscopy.

Introduction

Pullulan is a water soluble extracellular polysaccharide produced by strains of *Aureobasidium pullulans* consisting of a linear and flexible chain of D-glucopyranosyl units that alternate regularly between one α-(1,6) and two α-(1,4) linkages [1–4]. Owing to its oxygen impermeability, non-toxic and non-irritating properties, it is used for producing films binders, adhesives, thickeners, viscosity improvers, coating agents and gene delivery carriers [5,6]. Thus, pullulan has a number of potential applications, which may be improved and extended by introducing functional groups. To get tailored derivatives, chemical modification of the maltotriosyl unit of pullulan can be performed that contains 9 hydroxyl groups in a geometrically unique environment. Thus, products of a broad structural diversity may be synthesized [7,8]. Tezuka has performed synthesis of pullulan nonaacetate using acetic anhydride in pyridine and 4-dimethylamino pyridine [9]. Moreover, in a number of publications, in particular patents, it is described that a variety of pullulan derivatives is known, e.g., chlorinated- [10], chloroalkylated- [11], sulphinylethylated- [12], cyanoethylated- [13], carboxylated- [14], methylated- [15], cationized- [16], and sulphated pullulan [17].
Amphiphilic polymers undergo intramolecular microphase separation and form self-assembled aggregates with ordered supramolecular architectures. For instance, self-

assembly behavior of hydrophobized polysaccharides in water has been studied and it was found that cholesterol substituted pullulan derivatives were capable of forming gels and nano-particles [18,19] that might be of interest for pharmaceutical and biomedical applications [20-22]. Recently, amphiphilic polysaccharides (glyco-polymers) have attracted interest in the development of nano-sized devices, for improving interfacial biocompatibility, for studying carbohydrate-protein recognition and hemicellulose-cellulose interactions [23-27]. Hydrophobic abietic acid is extracted from tree resin, while pullulan is hydrophilic in nature [28]. It is already documented that abietic acid covalently bound on pullulan leads to amphiphilic products [29,30] (Fig. 1).

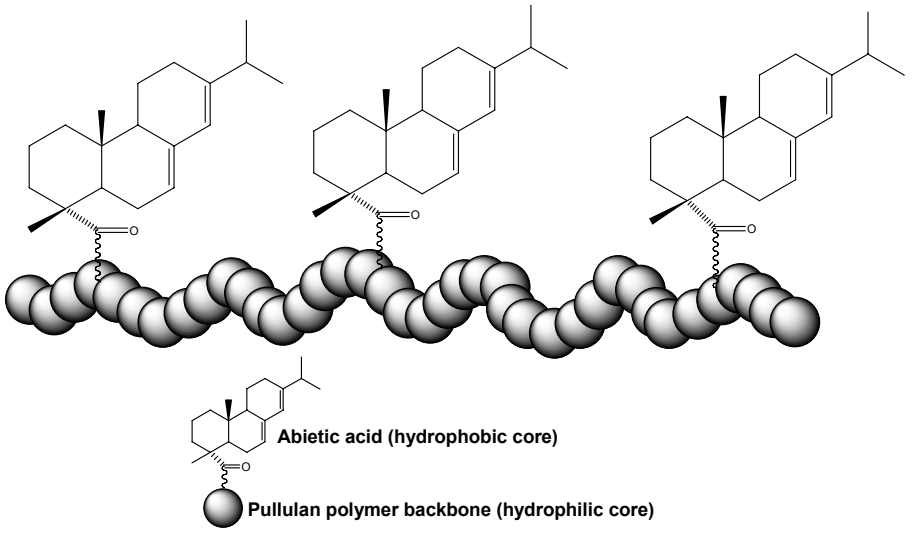

Figure 1. Schematic illustration of amphiphilic pullulan abietate

In the context of studies on the chemical modification of polysaccharides carried out at the Centre of Excellence for Polysaccharide Research, unconventional methods for pullulan acylation were studied. In the present paper, the synthesis of abietic acid esters of pullulan is discussed that was carried out homogeneously in *N,N*-dimethylacetamide (DMAc) by *in situ* activation of the carboxylic acid group with *p*-toluenesulfonyl chloride (Tos-Cl), *N,N`*-carbonyldiimidazole (CDI), and oxalyl chloride/*N*,*N*-dimethylformamide (DMF).

Experimental

Materials

Pullulan (70074) and abietic acid (Fluka) were dried under vacuum at 110°C for 8 h before use. All other chemicals supplied by Fluka were used without further purification.

Measurements

NMR spectra were acquired on a BRUKER Avance 400 (400 MHz) spectrometer applying DMSO-d_6. For ^{13}C NMR measurements (40°C) about 8,000 scans and for ^{1}H NMR 16 scans were accumulated. FTIR (KBr) spectra were measured on a Bio-Rad FTS 25 PC using pellet technique. Elemental analysis was performed with a CHNS 932 Analyzer (Leco). For gel permeation chromatography, an equipment of Agilent Technologies 1200 series was used including degasser (G1322A), pump (Quart Pump G1311A), RI-detector (RID G1362A) and Diode Array Detector (DAD G1315B) working at 360 nm. Deionised water was used as solvents (30°C, 1 mL/min). The separation was carried out using columns (Serial No. SM-MC-116-78) from Agilent Technologies UK with 5µm diameter. Polystyrene standards were used for calibration.

Dissolution of pullulan in N,N-dimethylacetamide (DMAc)

Pullulan (1 g) in 20 mL DMAc was stirred at 80°C for 30 min obtaining optically clear solution.

Synthesis of pullulan abietate with abietic acid/p-toluenesulfonyl chloride (Tos-Cl)

To the solution of 1.0 g (6.2 mmoles) pullulan in DMAc, 1.18 g (6.2 mmoles) Tos-Cl followed by 1.87 g (0.0062 moles) of abietic acid was added along with continuous stirring. The reaction mixture was stirred for 24 h at 70°C under N_2 and was precipitated in 200 mL ethanol (EtOH). The precipitate was washed with 200 mL EtOH three times and dried at 60°C under vacuum (product **1**).
Degree of substitution, DS: 0.06
Yield: 0.8 g
Elemental analysis (EA): 51.79% C, 7.26% H
FTIR (KBr): 3416 ν (OH), 2931 ν (C-H), 1724 ν (CO_{Ester}), 1246 ν (C-O-C_{Ester}) cm^{-1}.
^{13}C NMR (DMSO-d_6): δ=177.4, 170.5, 168.1 (CO), 101.7, 99.3, 96.0 (C-1_{ABC}), 80.4 (C-$4_{A, B}$), 60.9-73.8 (C-2, 3, 4_C, 5, 6_{AB}), 14.25 (C-26), 17.2-18.9 (C-25, 8), 21.1-27.5 (C-22, 23, 17, 12, 18), 34.6-38.3 (C-9, 7, 16, 21), 46.4 (C-10, 11), 50.9 (C-15), 120.8 (C-13), 122.9 (C-20), 134.9 (C-14) and 144.7 (C-19) ppm
^{1}H NMR (DMSO-d_6): δ = 3.26-5.27 AGU (anhydroglucose unit)-H, 0.73-2.75 (abietate moiety-H) and 5.71 (H-20) and 5.31 (H-13, overlapped with signals of AGU) ppm

Synthesis of pullulan abietate with abietic acid/ N,N`-carbonyldiimidazole (CDI)

To 1.86 g (6.2 mmoles) abietic acid dissolved in 30 mL DMF 1.5 g (6.2 mmoles) CDI was added and mixture was stirred over night. The mixture was added to 1.0 g (6.2 mmoles) pullulan dissolved in DMAc and allowed to react at 70°C for 24 h under stirring. The homogeneous mixture was precipitated in 300 mL EtOH and washed with 200 mL EtOH three times. The polymer was dried at 60°C under vacuum (pullulan ester **7**).
DS: 0.10
Yield: 0.7 g
EA: 43.38% C, 7.20% H.
FTIR (KBr): 3404 ν (OH), 2926 ν (C-H), 1724 ν (CO_{Ester}), 1244 ν (C-O-C_{Ester}) cm^{-1}
Other spectral data were comparable with that of sample **1**.

Synthesis of pullulan abietate with abietic acid/oxalyl chloride/N,N-dimethylformamide (DMF)

Abietic acid in 30 mL DMF was cooled to -20°C using dry ice and 0.59 mL (6.2 mmoles) oxalyl chloride was carefully added drop-wise. After gas-formation had stopped, 1.86 g (6.2 mmoles) abietic acid was added and mixed for 15 min. The mixture was added to the solution of 1.0 g (6.2 mmoles) pullulan in DMAc and stirred for 24 h at 70°C under N_2. The reaction mixture was precipitated in 300 mL acetone and the polymer was collected by filtration. After washing with 250 mL acetone three times, the polymer was dried at 50°C under vacuum to yield product **10**.
DS: 0.07
Yield: 0.7 g
EA: 40.83% C, 7.14% H
FTIR (KBr): 3411 ν (OH), 2929 ν (C-H), 1726 ν (CO_{Ester}), 1246 ν (C-O-C_{Ester}) cm^{-1}
Other spectral data were comparable with that of compound **1**.

Results and Discussion

Pullulan abietates (**1-12**) were homogeneously synthesized in *N,N*-dimethylacetamide (DMAc) using *in situ* activation of abietic acid with *p*-toluenesulfonyl chloride (Tos-Cl, method A), *N,N`*-carbonyldiimidazole (CDI, method B) and oxalyl chloride/ *N,N*-dimethylformamide (DMF, method C) at 70°C for 24 h (Fig. 2, Table 1). The formation mechanism of the intermediate, namely the mixed carboxylic acid-*p*-toluenesulfonic acid anhydride and the carboxylic acid chloride (method A), the carboxylic acid imidazolide (method B), and the carboxylic acid iminium chloride (method C), are discussed in detail in ref. [31].

Table 1. Conditions for and results of the reaction of pullulan dissolved in *N,N*-dimethylacetamide with abietic acid after *in situ* activation with *p*-toluenesulfonyl chloride (Tos-Cl, method **A**), *N,N`*-carbonyldiimidazole (CDI, method **B**), and oxalyl chloride/DMF (method **C**) at 70˚C for 24 h

Method	Molar ratio[a]	Sample	DS[b]	Yield (g/%)	Soluble in
A	1:0.25:0.25	**1**	0.06	0.8/66	Water, DMSO, DMAc
A	1:0.5:0.5	**2**	0.07	0.9/81	Water, DMSO, DMAc, DMF
A	1:0.75:0.75	**3**	0.10	1.0/68	Water, DMSO, DMAc
A	1:1:1	**4**	0.15	1.2/90	DMSO, DMAc, DMF
A	1:1.5:1.5	**5**	0.25	1.3/88	DMSO, DMAc, DMF, THF
A	1:2:2	**6**	0.46	1.4/75	DMSO, DMAc, DMF, THF
B	1:1:1	**7**	0.10	0.7/60	Water, DMSO, DMAc
B	1:1.5:1.5	**8**	0.14	0.8/63	Water, DMSO, DMAc
B	1:2:2	**9**	0.20	0.9/65	DMSO, DMAc
C	1:1:1	**10**	0.07	0.7/62	Water, DMSO, DMAc
C	1:1.5:1.5	**11**	0.10	0.9/76	Water, DMSO, DMAc
C	1:2:2	**12**	0.18	0.9/70	DMSO, DMAc

[a] = Anhydroglucose unit : acylation reagent : abietic acid
[b] = Degree of substitution, DS was calculated by acid-base titration after saponification

The degree of substitution (DS) of the samples was determined by acid-base titration after saponification. The DS of pullulan abietate obtained could be controlled by adjusting molar ratio of abietic acid to anhydroglucose unit (AGU). A product of high DS of 0.46 was synthesised by using 1 to 2 molar ratio of abietic acid to AGU (sample **6**). DS of 0.06 was obtained for sample **1**, which was synthesised using a molar ratio of 1:0.25:0.25 (AGU:Tos-Cl:abietic acid).

Pullulan abietates with DS ≤ 0.14 were soluble in water. With increasing DS the polymers become insoluble in water. Nevertheless, all products **1-12** were readily soluble in DMSO and DMAc. The water soluble products are of interest in the context to mimic cellulose-lignin interactions present in wood. Pullulan abietates were study easily adsorb onto regenerated cellulose surface over gold [27].

Success of reactions was established by FTIR spectroscopy, elemental analysis and NMR spectroscopic studies of samples. As a typical example, detailed structural characterization of sample **4** is discussed.

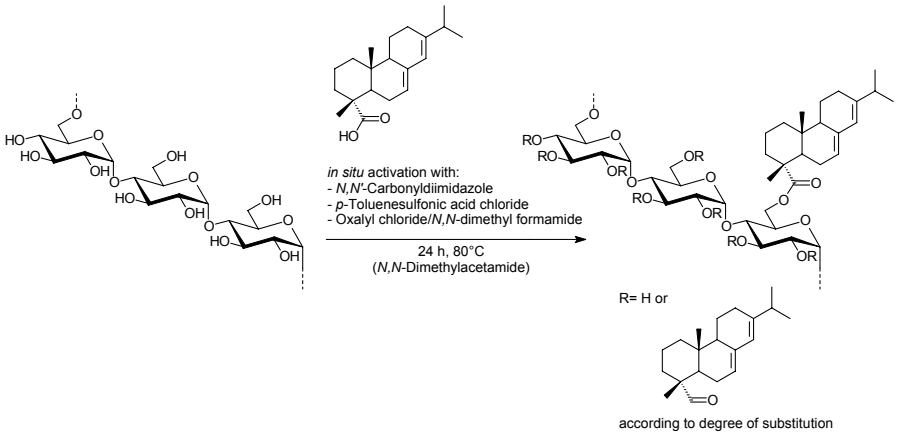

Figure 2. Schematic plot of the conversion of pullulan with abietic acid applying *in situ* activation.

FTIR (KBr technique) spectrum of **4** (Fig. 3) shows two characteristic peaks typical for the ester moieties appeared at about 1246 cm^{-1} (C-O-C_{Ester}) and about 1724 cm^{-1} (CO_{Ester}).

Elemental analyses revealed the absence of sulphur in the samples (**1-6**) prepared by applying Tos-Cl for the *in situ* activation (method **A),** hence no tosylate groups neither covalently bounded nor as impurity were introduced.

Typical ^{1}H NMR spectrum of **4** (DS 0.15) dissolved in DMSO-d_6 showed AGU signals of all protons of maltotriose units in the range of 3.26-5.5 ppm (AGU-H), protons of abietate moiety appearing in the range from 0.73 to 2.75 ppm. Protons at C=C of abietate appeared at 5.71 (H-20) and 5.31 (H-13, overlapped with AGU) ppm (Fig. 4). Due to the signal overlapping in ^{1}H NMR spectra, DS values can not be calculated.

A typical ^{13}C NMR spectrum of **4** recorded in DMSO-d_6 showed the characteristic ester-carbonyl peak at δ = 177.4, 170.5 and 168.1 ppm (Fig. 5) indicating that acylation with of abietic acid was not regioselective although the functional groups

possess a high steric demand. Signals of maltotriose unit of pullulan were found at δ = 101.7, 99.3, 96.0 ppm (C-$1_{A, B, C}$), for C-4 of ring **A** and B at 80.4 ppm (C-$4_{A, B}$) and for C-2, 3, 4_C, 5, 6 in the range of 60.9-73.8 ppm. Unsaturated carbons in abietic acid showed signals at δ = 120.8 ppm (C-13), 122.9 ppm (C-20), 134.9 ppm (C-14) and 144.7 ppm (C-19), which also confirmed the attachment of abietic acid group on to the pullulan backbone. NMR spectra of pullulan abietates were found to be comparable (maltotriose region) with the NMR spectra of pullulan nonaacetate [9].

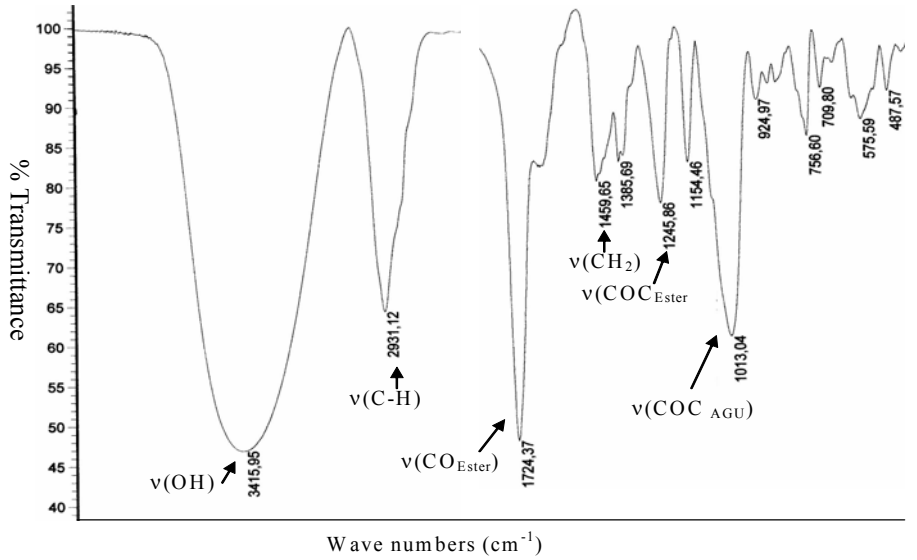

Figure 3. FTIR (KBr) spectrum of pullulan abietate **4**

Esterification of pullulan with abietic acid was also carried out using *in situ* activation of abietic acid with CDI. The imidazolide of the abietic acid is formed as reactive specie. DS 0.10 was achieved for sample **7** prepared by using molar ratio 1:1:1 (AGU:abietic acid:CDI) of the reactants. Sample **7** was water-soluble.

Another path for *in situ* activation was the conversion of carboxylic acid with iminium chlorides formed from oxalyl chloride/DMF. Iminium chloride reacted with pullulan to yield the corresponding ester. Pullulan abietate **10** with DS 0.07 showed the carbonyl ester peak at 1726 cm^{-1} in the FTIR (KBr) spectrum.

Comparing the methods mentioned above for the acylation of pullulan with abietic acid, it was found that Tos-Cl (method A) is more efficient and higher DS values were obtained as compared to CDI (method B) and iminium chloride (method **C**). Under comparable conditions, a DS of 0.46 (sample **6**, method A), DS 0.20 (**9**, method B) and DS 0.18 (**12**, method **C**) was obtained applying at a molar ratio of 1 : 2 : 2 (AGU:abietic acid:acylation reagents).

GPC studies of pullulan abietates in aqueous solution revealed a degree of polymerization (DP_n) of 19 (**1,** DS 0.06), 137 (**7**, DS 0.10) and 142 (**10**, DS 0.07) indicating that *in situ* CDI and iminium chloride was milder compared to conversion via *in situ* activation with tosyl chloride (Fig. 6) It must be pointed out that the activation with oxalyl chloride/DMF yielding the corresponding iminium chloride

may lead to a strong degradation if water is present. Therefore, a strictly water-free reaction system is recommended to keep the DP at a high value.

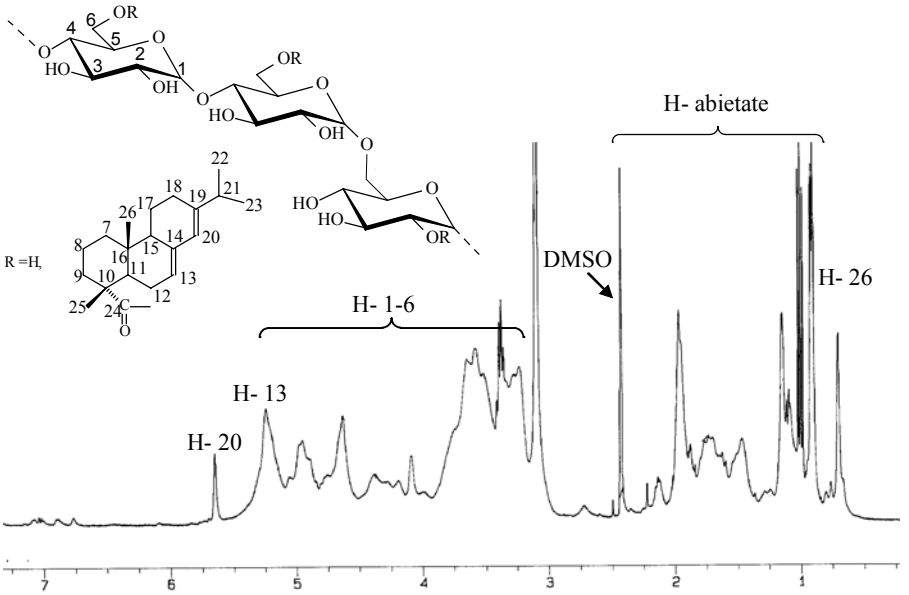

Figure 4. ^{1}H NMR spectrum (DMSO-d_6, 16 scans, values in ppm) of pullulan abietate **4**

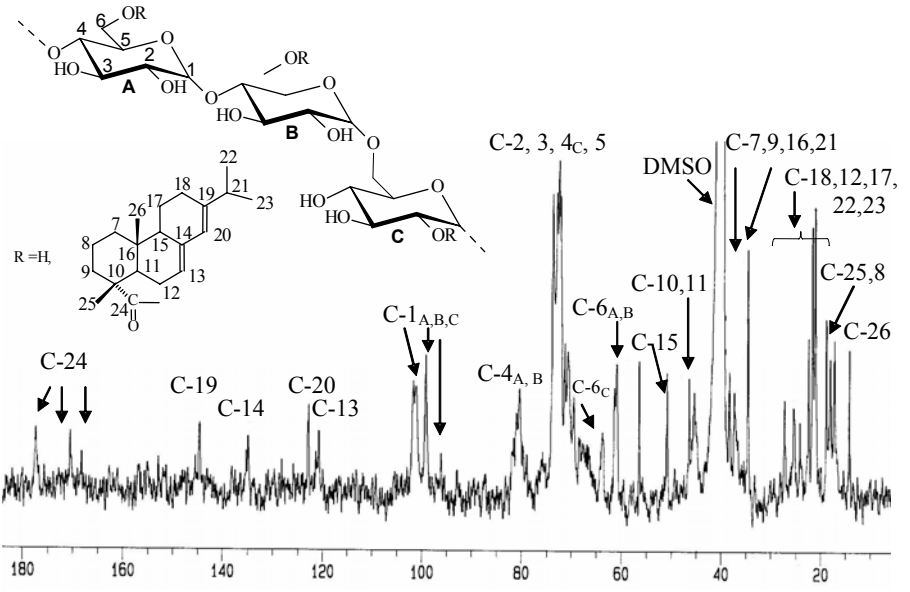

Figure 5. ^{13}C NMR spectrum (DMSO-d_6, 7934 scans, values in ppm) of pullulan abietate **4**

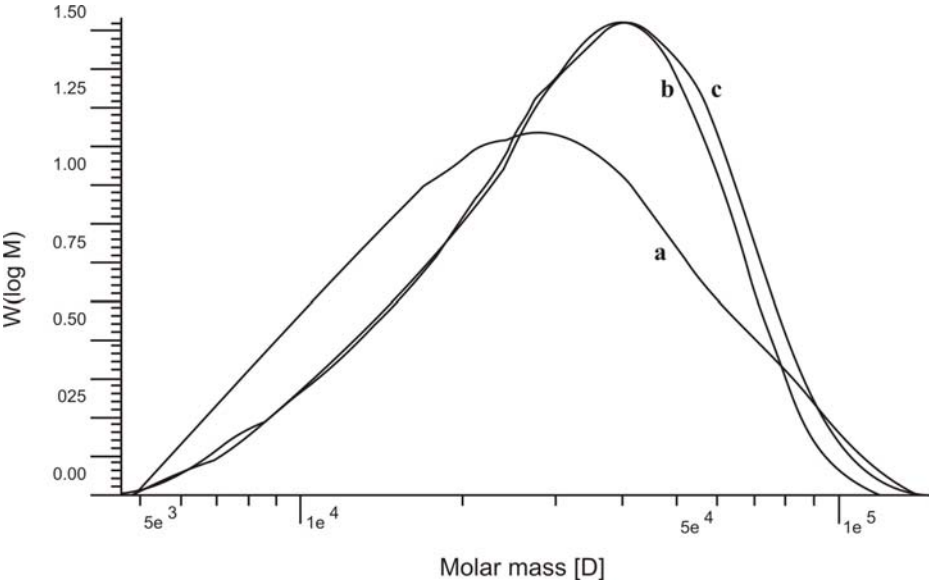

Figure 6. Gel permeations chromatograms of of pullulan abietate of) a sample **1** (degree of substitution, DS 0.06), b) **7** (DS 0.10), and c) **10** (DS 0.07)

Conclusions

Pullulan abietates with different degree of substitution (DS) could be prepared homogeneously in DMAc via *in situ* activation of abietic acid with *p*-toluenesulfonyl chloride, *N,N'*-carbonyldiimidazole and iminium chloride formed from oxalyl chloride/DMF. It clearly appears the in situ activation with Tos-Cl is most efficient; the highest DS values were realized, on one hand. On the other, *in situ* activation with CDI and with iminium chloride (provided a water-free reaction mixtures is guaranteed) gave products of high DP, i.e., there is almost no degradation. Samples with a DS ≤ 0.14 were soluble in water; at higher DS solubility in organic solvents appeared only.

Acknowledgements. Muhammad Ajaz Hussain thanks Dr. T. Liebert for helpful discussion about polysaccharide chemistry. Thomas Heinze gratefully acknowledges the general financial support of the "Fonds der Chemischen Industrie", Germany.

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