

# Chemical Modification of Beechwood Xylan with *p*-Carboxybenzyl Bromide

A. EBRINGEROVÁ,\* Z. NOVOTNÁ, M. KAČURÁKOVÁ, and E. MACHOVÁ

Institute of Chemistry, Slovak Academy of Sciences, SK-84238 Bratislava, Slovak Republic

## SYNOPSIS

Xylan isolated from beechwood was alkylated with *p*-carboxybenzyl bromide in aqueous alkali. The alkylation by varying stoichiometry and reaction temperature yielded carboxybenzylated xylan derivatives with a degree of substitution which ranged from 0.05 to 0.25. The reaction products were characterized by elementary analysis and FTIR and UV spectroscopy. As tested by HPGPC, no degradation of the xylan chains resulted up to a reaction temperature of 40°C. The derivatives show moderate surface activity. © 1996 John Wiley & Sons, Inc.

## INTRODUCTION

Next to cellulose, xyans represent the most abundant class of naturally occurring organic compounds and an important renewable resource of plant polymers. They exist in many structural variations<sup>1</sup> whose potential has not yet been completely realized. In the last decade, many useful applications of xyans as crude mixtures or relatively pure polymers have been reviewed.<sup>2,3</sup> Chemical modification of xyans gave rise to novel molecular functions applicable to the preparation of antitumor, antiviral, and antibacterial agents, paper additives, detergents, photosensitive materials, adhesives, flocculants, etc.<sup>2,4</sup>

In this article, we report on the preparation of a novel 4-*O*-methylglucuronoxylan derivative containing *p*-carboxybenzyl substituents. Emphasis was put on reaction conditions that minimize chain degradation.

## EXPERIMENTAL

### Materials and Chemicals

The 4-*O*-methyl-D-glucurono-D-xylan from beechwood (GX) was prepared<sup>5</sup> on a semitechnical scale

in our institute. Its analytical characteristics are given in Table I. *p*-Bromomethylbenzoic acid (BBA) was prepared according to ref. 7. UV<sub>254</sub>-sensitive Silicagel plates (Silufol) were purchased from Kavalier (Brno, Czechoslovakia).

### Analytical Methods

The methods used for characterization of GX were described in previous articles.<sup>8,9</sup> The moisture content was determined by drying the sample to a constant weight at 60°C *in vacuo*. The elementary analysis was performed on an elemental analyzer Model 240 (Perkin-Elmer). For calculations of theoretical carbon and hydrogen contents, the formula for GX (H<sup>+</sup> form) with a uronic acid content of 12.4% was used: [C<sub>5.7</sub>H<sub>9-DS</sub>O<sub>4.6</sub> · (C<sub>7</sub>H<sub>8</sub>O<sub>2</sub>)<sub>DS</sub>]. The presence of unreacted BBA and its degradation products in the reaction medium was tested by TLC on a Silufol using toluene–ethylacetate (7 : 2, v/v) as the developer and UV light for the detection. Samples of the original and modified GX were passed into H<sup>+</sup> form by treating them with acidified 90% ethanol containing 0.1 mol L<sup>-1</sup> of HCl, and their carboxyl group content was determined by potentiometric titration with aqueous 0.05M NaOH.<sup>10</sup>

FTIR spectra were measured in KBr pellets (2 mg sample/200 mg KBr) using a Nicolet-Magna 750 spectrophotometer operating at 4 cm<sup>-1</sup> resolution. The peak absorbance ratio method was applied to

\* To whom correspondence should be addressed.

**Table I Analytical Characteristics of the Beechwood Xylan GX**

Moisture (%)	9.2
Ash (sulfate) (%)	2.1
Neutral sugars (rel. wt %)	
D-Xylose	95.6
D-Glucose	3.7
D-Mannose	0.5
D-Galactose	1.4
Uronic acid, (%) <sup>a</sup>	12.4
$[\alpha]_D$ in 1% NaOH ( $c = 0.5$ ) (degrees)	-63
$[\eta]^b$ (cm <sup>3</sup> g <sup>-1</sup> )	46
Solubility in cold water (%)	79

<sup>a</sup> As anhydrounit of 4-*O*-methyl-D-glucuronic acid;  $M_r = 190$ .

<sup>b</sup> Measured in DMSO.<sup>6</sup>

the spectra with a base line at 800 and 1520 cm<sup>-1</sup>. UV spectra were recorded with a Pye-Unicam 1700 UV spectrophotometer and *p*-toluic acid (PTA) in 80% ethanol was used as the calibration standard.

HPGPC of GX and its carboxybenzyl (CB) derivatives was performed using a commercial instrument (Laboratorní přístroje, Prague, Czechoslovakia) equipped with a Separon HEMA-BIO 100 and 1000 exclusion columns (Tessek Ltd., Prague) and 0.1M aqueous NaNO<sub>3</sub> solution as the solvent and eluent. The eluate was monitored by refractometry

and UV absorption at 254 nm. The columns were calibrated with a set of Pullulan standards (Shodex Standard P-82, Macherey-Nagel GmbH, Germany). The computing procedure<sup>11</sup> based on the linear effective calibration curve was applied to obtain the molecular weight distribution.

#### Preparation of *O*-(*p*-carboxybenzyl)xylan (CBGX)

In a typical experiment, GX (1 g) was dispersed in water containing 0.6 g NaOH and the dispersion was stirred at room temperature for 1 h. Then, BBA (3.2 g) was added and the stirring was continued until the disappearance of BBA in the reaction medium (tested by TLC). Finally, the pH was brought to 8.0 with NaOH and the reaction product was dialyzed to pH 7. The nondialyzable portion was recovered by evaporation under reduced pressure and dried over P<sub>2</sub>O<sub>5</sub>. A sample of the derivative (CBGX) was dispersed in water (0.5 g/50 mL) and stirred at room temperature for 4 h. The soluble portion was separated by centrifugation and recovered by freeze-drying (ws-CBGX).

#### Interfacial Properties

The interfacial properties of CBGX derivatives were assessed by a surface-tension measurement using a

**Table II Yield and Analytical Data for Some *O*-(*p*-carboxybenzyl)glucuronoxylan Derivatives**

Sample	BBA/GX <sup>a</sup>	Yield (g/g)	$A_{1548}/A_{1462}$ (cm <sup>-1</sup> /cm <sup>-1</sup> )	C (% calcd) (% found)	H (% calcd) (% found)	DS
CBGX1	1 <sup>b</sup>	1.15	0.87	ND	ND	0.12 <sup>c</sup>
CBGX2	2 <sup>b</sup>	1.10	0.94	ND	ND	0.15 <sup>c</sup> 0.14 <sup>d</sup>
CBGX3	3 <sup>b</sup>	1.20	0.96	48.39 48.12	5.78 5.81	0.15 <sup>e</sup> 0.17 <sup>d</sup>
CBGX4	1 <sup>f</sup>	0.95	0.79	47.06 46.85	5.88 5.90	0.10 <sup>e</sup> 0.12 <sup>c</sup>
CBGX5	1 <sup>g</sup>	0.87	0.55	46.42 46.51	5.90 5.82	0.05 <sup>c</sup>
ws-CBGX1	1 <sup>b</sup>	0.75	1.34	49.76 49.47	5.71 5.65	0.23 <sup>e</sup> 0.25 <sup>c</sup> 0.22 <sup>d</sup>

ND, not determined.

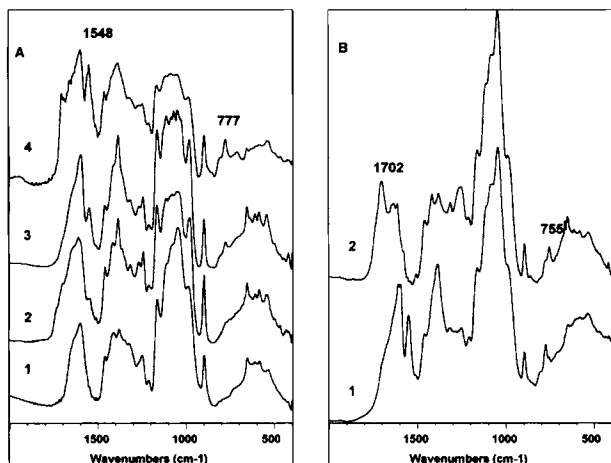
<sup>a</sup> In mol/132 g GX; NaOH/GX: 1-3 mol/132 g.

<sup>b</sup> Reaction temperature: 20°C.

<sup>c-e</sup> DS calculated from <sup>c</sup> UV<sub>240</sub>, <sup>d</sup> potentiometric titration, and <sup>e</sup> elementary analysis.

<sup>f</sup> Reaction temperature: 40°C.

<sup>g</sup> Reaction temperature: 60°C.



**Figure 1** (A) FTIR spectra of the Na<sup>+</sup> form of (1) GX and (2) CBGX derivatives; DS = 0.10; (3) DS = 0.15; (4) DS = 0.23. (B) FTIR spectra of the (1) protonated and (2) salt form of ws-CBGX1.

DuNouy ring apparatus. The critical micelle concentration ( $c_0$ ) was calculated from the plot of the surface tension data against the log of concentration.

## RESULTS AND DISCUSSION

Reaction of GX with BBA under alkaline conditions results in the nucleophilic substitution of the xylan hydroxyl groups. The reaction was monitored by the disappearance of BBA in the reaction medium. In preliminary experiments performed in the absence of GX, it was shown that in 1% NaOH (at room temperature) the decomposition of BBA was complete after about 96 h, whereas it needs only 4 h in 4% NaOH (data not shown). To minimize degradation of the xylan chain, the modification of GX was performed in 1% NaOH. Following the reaction procedure at various reaction conditions, a series of CBGX products differing in yields and elementary analysis data were prepared (Table II).

The FTIR spectra of the salt form of the parent and modified GX are displayed in Figure 1 (A). The introduction of CB groups in the xylan chain gives rise to new absorption bands at  $\sim 1548$  and  $777\text{ cm}^{-1}$  which increase with increasing degree of substitution (DS). These bands are attributed to  $\nu_{\text{as}}(\text{COO}^-)$  and benzene ring vibrations of the CB substituent based on published IR data of BBA<sup>12</sup> and *N*-(*o*-carboxybenzylidene) chitosan.<sup>13</sup> The other ring absorption band of BBA at  $\sim 1610\text{ cm}^{-1}$  is in CBGX overlapped by the  $\nu_{\text{as}}(\text{COO}^-)$  of the uronic

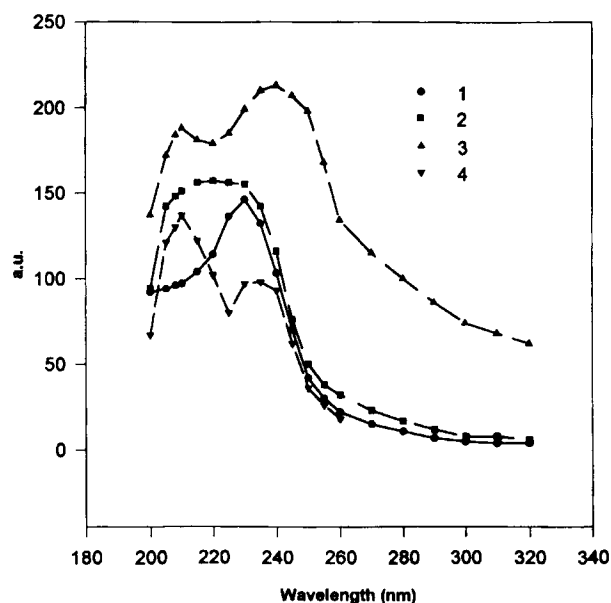
acid GX constituents.<sup>14,15</sup> In going from the salt to the acid form of the CBGX carboxyl groups, remarkable changes in the IR spectra are observed [Fig. 1 (B)]. The  $\nu(\text{C}=\text{O})$  vibration of the aromatic carboxyl group appears at  $1702\text{ cm}^{-1}$ . The (CH) bending perpendicular to the plane of the benzene ring is shifted from  $777\text{ cm}^{-1}$  toward a lower wave number ( $755\text{ cm}^{-1}$ ). The assignments of the spectral bands of CBGX are summarized in Table III. The IR spectra, therefore, confirm the presence of carboxybenzyl groups in the modified xylan and should give information on the form of both present carboxyl groups. However, the salt form of CBGX is more convenient for analytical purposes.

The UV spectra of CBGX at various pH and PTA are displayed in Figure 2. The CBGX solution brought to pH 3 with HCl or to pH 12 with NaOH present two absorption maxima at 210 and 230–240 nm, similarly to PTA in methanol. Only one maximum at 230 nm occurs at neutral conditions. The absorption band at 240 nm and calibration with PTA were used to determine the DS of CBGX derivatives. The obtained values agree well with the DS calculated from the elementary analysis and potentiometric

**Table III** Assignment of the Main Bands in FTIR Spectra of CBGX Derivatives

Frequency Range (cm <sup>-1</sup> )	Band Assignment	Origin
1730–1723	$\nu(\text{C}=\text{O})$	COOH of UA
1705–1699	$\nu(\text{C}=\text{O})$	COOH of CB group
$\sim 1634$	$\delta(\text{HOH})$	Adsorbed water
$\sim 1612$	ring	CB group
$\sim 1600$	$\nu_{\text{as}}(\text{COO}^-)$	COONa of UA
1578	ring	CB group
1551–1545	$\nu_{\text{as}}(\text{COO}^-)$	COONa of CB group
$\sim 1510$	ring	CB group
1467–1453	$\delta(\text{CH})$	Xylan
1419–1410	$\nu_{\text{s}}(\text{COO}^-)$	COONa of UA
1390–1385	$\nu_{\text{s}}(\text{COO}^-)$	COONa of CB group
$\sim 1382$	$\delta(\text{CH})$	Xylan
$\sim 1261$	$\nu(\text{C}-\text{O}),$ $\delta(\text{CH})$	CB group
$\sim 1113$	ring	CB group
1045	$\nu(\text{C}-\text{O}),$ $\nu(\text{C}-\text{C})$	Xylan
897	$\delta(\text{C}-1-\text{H})$	Xylan
$\sim 777$	$\delta(\text{CH}),$ ring	CB (Na <sup>+</sup> form)
$\sim 757$	$\delta(\text{CH}),$ ring	CB (H <sup>+</sup> form)

$\nu$ , stretching;  $\delta$ , bending; as, asymmetric; s, symmetric vibrational modes; UA, 4-*O*-methyl-D-glucuronic acid.



**Figure 2** UV spectra of ws-CBGX1 in water at (1) pH 3, (2) pH 12, (3) pH 7, and (4) of PTA in methanol.

metric titration as well (Table II). The DS show a direct correlation with the absorption band intensity ratio  $A_{1548}/A_{1462}$  derived from the IR spectra of CBGX (curve not shown), suggesting the valuability of the FTIR spectroscopy for CBGX analysis.

The data in Table II show that the DS of the studied CBGX derivatives ranged from 0.05 to 0.15. Only a slight increase of DS was observed by increasing the proportion of both NaOH and BBA at ambient temperature. At higher reaction temperatures, the DS has a decreasing tendency, suggesting that side reactions of BBA are favored. The CBGX preparations are partially soluble in water. The DS of the water-soluble part of CBGX1 has a twofold higher value (0.23) compared to that of the unfractionated product. The DS of the water-soluble parts of the other CBGX samples varied in the range of 0.15–0.25 (data not shown). The results indicate that the alkylation reaction proceeded nonuniformly, probably due to the limited solvation of GX in the dilute alkali.

The yields of CBGX varied from 1.0 to 1.2 g CBGX/g GX up to the reaction temperature of 40°C (Table II). At higher temperatures, the yield decreased, indicating degradation of the xylan chain. This was supported by the results of HPGPC analysis of GX and its CB derivatives performed on Separon HEMA BIO gels. By monitoring the elution with RI and UV<sub>254</sub> detectors, two elution curves were obtained which exhibit the same pattern, which

**Table IV** Molecular Weight Distribution<sup>a</sup> of GX and CBGX Derivatives

Sample	$M_w \times 10^{-3}$	$M_w/M_n$	Area %
GX	35.5	2.01	98
	161.0	—	2
CBGX1	37.1	1.72	97
	168.1	1.08	3
CBGX2	38.0	1.52	98
	171.0	1.01	2
CBGX4	36.2	1.61	94
	163.0	1.10	6
CBGX5	32.3	2.13	92
	160.0	1.05	8

<sup>a</sup> Determined by HPGPC on Separon HEMA-BIO exclusion columns.

suggests alkylation in the whole molecular weight range. As seen in Table IV, substantial changes of the molecular weight distribution and a decrease of the weight-average molecular weight ( $M_w$ ) occur only at elevated reaction temperatures, particularly above 40°C.

The interfacial properties of two samples differing in DS (0.13 and 0.24) were assessed by surface-tension measurements. Both derivatives lower the surface tension of water from 71.5 to 65.1 and 54.4 mN m<sup>-1</sup>, corresponding to  $c_0 \sim 3.5$  and 2.3 g L<sup>-1</sup>, respectively. The results (Table V) indicate a rather weak surface activity of the xylan derivatives which is comparable with that reported for tamarind polysaccharide and its alkylated derivative bearing short alkyl chains.<sup>16</sup> Further studies on the interfacial properties of the novel xylan derivative are in progress.

**Table V** Surface Tension of CBGX Derivatives and Other Polysaccharides (0.1% Aqueous Solutions)

Sample	Surface Tension (Air/Water) (mN m <sup>-1</sup> )	Refs.
H <sub>2</sub> O	71.5	15
Tamarind/H <sub>2</sub> O	61.3	15
Octyl tamarind/H <sub>2</sub> O	55.6	15
CBGX (DS = 0.13)/H <sub>2</sub> O	65.1	<sup>a</sup>
CBGX (DS = 0.24)/H <sub>2</sub> O	54.4	<sup>a</sup>

<sup>a</sup> This article.

## CONCLUSIONS

O-(*p*-Carboxybenzyl)glucuronoxylan derivatives with DS up to 0.25 can be prepared under mild reaction conditions. A significant degradation of the xylan molecule was observed only at higher reaction temperatures. CBGX is soluble in water in its carboxylated form and gives transparent solutions. At acidic conditions, coagulation takes place. CBGX exhibits different IR spectra depending on the degree of protonation. The identification of CBGX by FTIR spectroscopy is unambiguous only when measured in the salt form. The DS of CBGX and its concentration in solution can be easily determined by UV spectroscopy using the absorption band at 240 nm.

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