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Selective oxidation of primary alcohol groups of β-cyclodextrin mediated by 2,2,6,6-tetramethylpiperidine-1-oxyl radical (TEMPO)

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Abstract

β-Cyclodextrin (β-CD) was reacted with catalytic amounts of 2,2,6,6-tetramethylpiperidine-1-oxyl radical (TEMPO), sodium hypochlorite and sodium bromide at 2 °C and a pH value of 10 in water. The primary alcohol groups were selectively oxidized into carboxylate groups within a few minutes, and mono- and dicarboxy-β-cyclodextrin sodium salts were isolated and characterized by ^{1}H, ^{13}C NMR and mass spectroscopy. With this reaction system, the degradation of the cyclodextrin was limited, provided the oxidation was performed at 2 °C, at constant pH value of 10, with catalytic amounts of TEMPO and controlled quantities of sodium hypochlorite and sodium bromide for the continuous regeneration of the oxoammonium salt. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: TEMPO oxidation; β-Cyclodextrin; Primary alcohol oxidation; 6-Carboxy-β-cyclodextrins; ^{1}H and ^{13}C NMR

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1. Introduction

Cyclodextrins are a family of cyclic α-(1 → 4)-linked oligosaccharides consisting mainly of six, seven and eight glucose units. They have the remarkable property of complexing with a large variety of guest molecules in their hydrophobic cavity. Selective chemical modifications of the hydroxyl groups in order to prepare new compounds with specific properties are an interesting but difficult challenge due to the large number of hydroxyl groups. Numerous modified cyclodextrins were reported, and several efficient methods for the selective modification of primary hydroxyl groups were described [1,2]. The introduction of one or several carboxylic groups is interesting, in particular, for increased association with cationic substrates, better solubility in water and immobilization on polymer support. Selective oxidation of β-cyclodextrin (β-CD) with platinum-charcoal has already been described [3] to produce monocarboxy-β-CD. Monoaldehydic cyclodextrins have already been synthesized according to different routes, and they can be oxidized into the corresponding carboxylic acids [4–9], but all these different reaction procedures are time consuming and difficult to control. Only recently organic nitroxyl radicals have been used for the selective oxidation of primary alcohols of carbohydrates [10]. The catalytic effect of bromide in the hypochlorite oxidation of starch and inulin without 2,2,6,6-tetramethylpiperidine-1-oxyl radical (TEMPO) led to dicarboxy-starch

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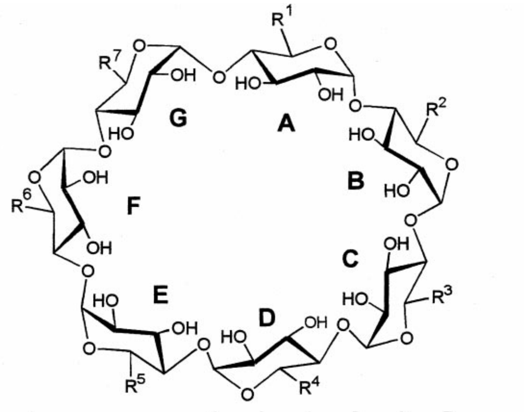
[11] and dicarboxy-inulin [12]. De Nooy et al. [13,14] described a method for the selective oxidation of primary alcohol groups of water-soluble polysaccharides (i.e., starch, amylodextrin and pullulan) with hypochlorite, sodium bromide and catalytic amounts of TEMPO. By applying the same TEMPO–NaBr–NaClO system to water-insoluble polysaccharides, i.e., cellulose and chitin, Chang and Robyt [15] and Isogai and Kato [16] obtained polymers with primary alcohol groups selectively oxidized.

In this work we studied the oxidation of primary hydroxyl groups of β-CD with sodium hypochlorite, sodium bromide and catalytic amounts of TEMPO. When the pH was maintained at 10, the primary alcohol groups were selectively oxidized, and a mixture of the different isomers of carboxylated cyclodextrins was obtained depending on the quantities of sodium hypochlorite introduced. The isolation and analysis of mono- and dicarboxy-cyclodextrins were also described.

2. Results and discussion

The β-CD was oxidized in the presence of TEMPO using sodium hypochlorite and sodium bromide following the protocol described by Vignon et al. [17] and also reported in the Section 3. During the reaction, the pH was maintained at the desired pH 10 by addition of 0.5 M sodium hydroxide solution to prevent oxidation of secondary hydroxyls. Under these conditions, primary alcohols were selectively and rapidly oxidized and a mixture of mono- to heptacarboxy-β-CDs was obtained and fractionated on biogel P6 column eluted with sodium nitrate solution. Four fractions were isolated corresponding, respectively, fraction 1 (F1) to the unreacted β-CD, fraction 2 (F2) to the monocarboxy-β-CD, fraction 3 (F3) to the dicarboxy-β-CD and fraction 4 (F4) to a mixture of tri, tetra,…, heptacarboxy-β-CD. From theoretical consideration on the number and possible structures of the isomers of oxidized β-CDs, there is only one product when one, six or seven carboxyl groups are formed. Three possible isomers can be produced when two or five carboxyl groups are formed; five possible isomers can be produced when three or four primary hydroxyl groups are oxidized. In this paper we report mainly on monocarboxy-β-CD sodium salt (compound **1**), and briefly on the mixture of dicarboxy-β-CD sodium salts (compounds **2**–**4**). The identity and structure of **1** and compounds **2**–**4** were confirmed by NMR and mass spectrometries; however, the three isomers **2**–**4** were not separated (Fig. 1).



1 $R^1 = COONa$; $R^2 = R^3 = R^4 = R^5 = R^6 = R^7 = CH_2OH$

2 $R^1 = R^2 = COONa$; $R^3 = R^4 = R^5 = R^6 = R^7 = CH_2OH$

3 $R^1 = R^3 = COONa$; $R^2 = R^4 = R^5 = R^6 = R^7 = CH_2OH$

4 $R^1 = R^4 = COONa$; $R^2 = R^3 = R^5 = R^6 = R^7 = CH_2OH$

Fig. 1. Structure of β-CDs mono- and dicarboxylated at C-6 with glucose rings label A–G.

The β-CD presented very simple ^1H and ^{13}C spectra at 500 MHz, due to the sevenfold rotational symmetry of the system. As expected, mono and disubstituted β-CDs lack their symmetry and therefore exhibit a more complex spin system. Indeed, in the proton spectrum of compound **1** we can observe in the anomeric region, the presence of six overlapping doublets between δ 4.94 and 4.98, and of one well individualized doublet at δ 4.91, with $^3J_{H-1,H-2}$ 3.5 Hz. A doublet (^3J 9.75 Hz) at δ 3.97 is characteristic of H-5 of residue A, which has been monooxidized at the 6-position and bears the carboxylate group. By carrying out 1D TOCSY experiments with a short mixing time after selective excitation of H-5A, the magnetization of the H-5A proton transfers only to H-4A. With increased mixing times the magnetization transfers to more distant protons (H-3A, H-2A, H-1A), and we can assign all the proton signals of unit A

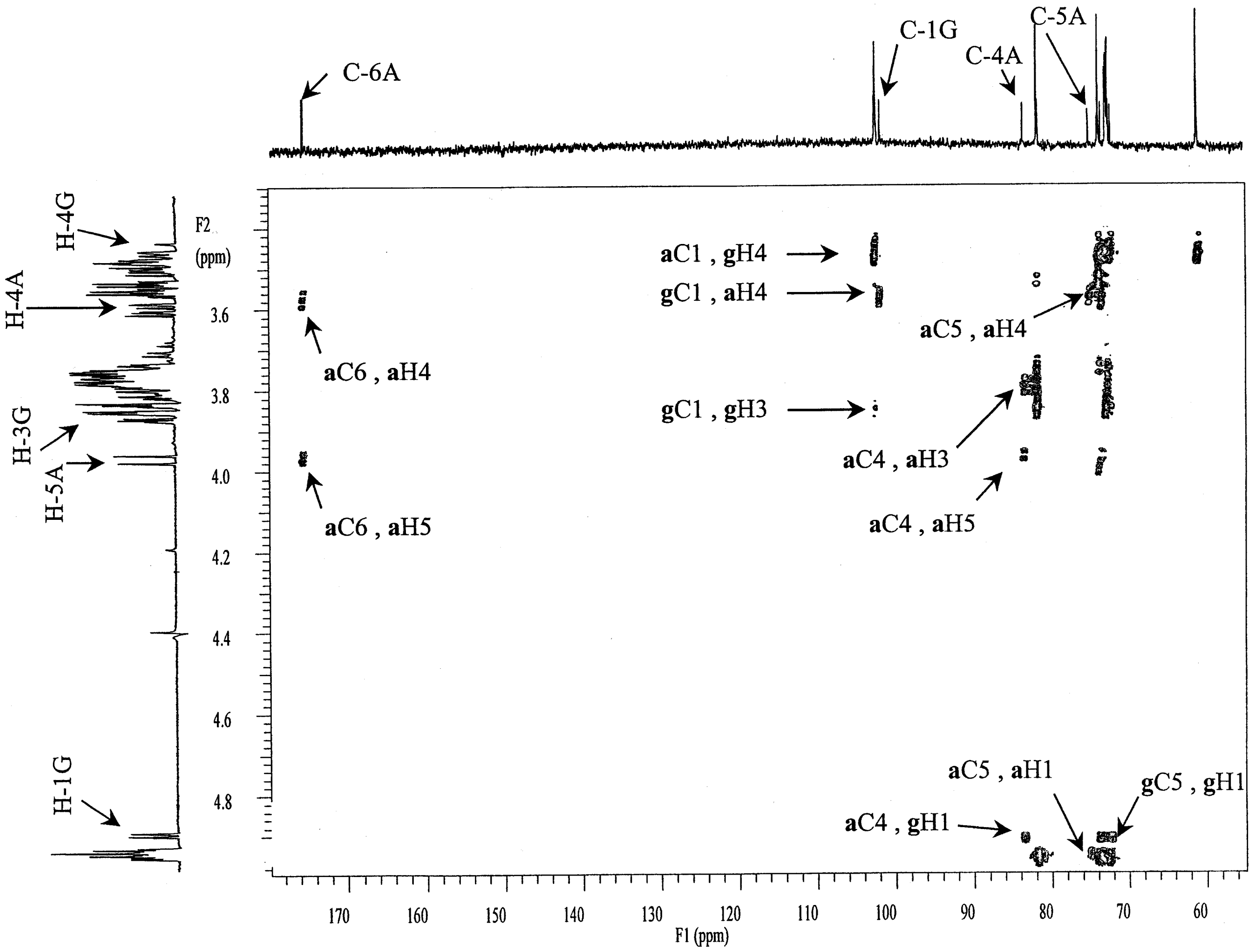


Fig. 2. 500 MHz 2D ^{13}C–^{1}H HMBC spectrum of compound **1** recorded in D$_2$O at 50 °C. The code **a**C-6, **a**H-5 indicates a long-range coupling between C-6 of unit A and H-1 of unit A. The code **a**C-4, **g**H-1 indicates a long-range coupling between C-4 of unit A and H-1 of unit G across the glycosidic bond.

(4.95, H-1A, 3.59, H-2A, 3.79, H-3A, 3.57, H-4A, 3.97, H-5A). The same 1D TOCSY experiment performed on H-1 at δ 4.91 allows the following assignment of protons at δ 3.49, H-2; 3.825, H-3; 3.44, H-4; 3.65–3.75, H-5, H-6a and H-6b either to unit B or G. In the ^{13}C spectrum of compound **1** we can observe the presence of at least 30 well individualized signals, with characteristic peaks easily assigned by 2D experiments. In the 2D ^{13}C–^{1}H HMBC spectrum (Fig. 2), intra-residual two- and three-bond ^{13}C,^{1}H couplings were observed, in addition to inter-residual three-bond connectivities over the glycosidic linkages. Strong cross-peaks between C-6 and H-5 of unit A and C-6 and H-4 of unit A confirm the presence of a carboxylic group at C-6 of unit A at δ 175.50. Strong connectivities between C-1G and H-4A, C-4A and H-1G, C-4A and H-5A and C-5A and H-4A demonstrated that the well individualized signal at δ 4.91 corresponded to H-1G, and thus the above proton signals assigned either to unit B or G can be unambiguously assigned to residue G at δ 3.49, H-2G; 3.825, H-3G; 3.44, H-4G; 3.65–3.75, H-5G, H-6aG and H-6bG. We can also deduce that carbon peaks at δ 102.42, 102.00, 83.63 and 75.19 can be assigned to C-1A, C-1G, C-4A and C-5A, respectively.

The proton spectrum of fraction F3 showed in the anomeric region, the presence of numerous overlapping doublets between δ 4.94 and 5.0, and of six well-individualized doublets between δ 4.86 and 4.94, with $^{3}J_{\text{H-1,H-2}}$ 3.5 Hz. Moreover, we can observe the presence of six overlapping doublets (^{3}J 9.75 Hz) between δ 3.89 and 4.04, characteristic of protons H-5 of dicarboxy-cyclodextrins [regioisomers AB (**2**), AC (**3**) and AD (**4**)].

The FABMS spectrum of compound **1** showed a molecular ion $[M+H]^+$ at *m*/*z* 1171 and an important molecular ion $[M+Na]^+$ at *m*/*z* 1193. These signals are consistent with the presence of one sodium carboxylate group in the molecule. The FABMS spectrum of fraction F3 (compounds **2**–**4**) showed a molecular ion $[M-Na+H]^+$ at *m*/*z* 1185 and strong molecular ions $[M+H]^+$ and $[M+Na]^+$ at *m*/*z* 1207 and 1229, respectively. These signals are consistent with the presence of two sodium carboxylate groups in compounds **2**–**4**.

The primary alcohol groups were selectively oxidized, more likely because the reaction involves the sterically hindered 2,2,6,6-tetramethyl-1-piperidine oxoammonium ion. The primary alcohol groups were oxidized in carboxylate groups, but mixtures from mono- to heptacarboxy-cyclodextrins with varying relative amounts were obtained. In this paper we studied the effects of the amount of NaBr and NaClO added on the resultant yields of the products. As shown in Table 1, the better yield in monocarboxy-β-CD was obtained with 0.5 mmol of NaBr and 1.0 mmol of NaClO per mmol of primary alcohol. The better yield in dicarboxy-β-CD was obtained with 0.8 mmol of NaBr and 1.6 mmol of NaClO per mmol of primary alcohol. Monocarboxy-β-CD can be obtained in 26% and dicarboxy-β-CD in 31% by direct oxidation of the β-CD, and these yields in mono- and dicarboxy-β-CDs were much higher than the ones already reported in the literature [9]. The oxidized carboxy-β-CD presented a greater solubility in water than β-CD. Inclusion experiments with mono- and dicarboxy-cyclodextrins are in progress.

3. Experimental

General methods.—The 1D and 2D NMR experiments were recorded on a Varian UNITY plus 500 spectrometer (^1H frequency of 500.619 MHz, and ^{13}C frequency of 125.890 MHz), using the VARIAN standard pulse sequences. The delay time for the detection of long-range $^{13}C-^1H$ couplings was set to 120 ms in HMBC experiments. Samples were studied as solutions in D_2O (5 mg in 2 mL of solvent) at 50 °C in 5 mm o.d. tubes. HOD signal (δ 4.40) was used as an internal standard. ^1H spectra were recorded using 90° pulses, 3300 Hz spectral width, 12,480 data points, 1.891 s acq. time and 32 scans were accumulated. ^{13}C NMR experiments were obtained on the same spectrometer and on the same tube (internal acetone ^{13}C (CH_3) δ 31.5 relative to Me_4Si). The FABMS spectra in the positive mode were recorded on a R 1010C quadripolar mass spectrometer (model 2000, Nermag, Reuil-Malmaison, France), equipped with a M Scan Wallis-type gun (8 kV, 20 mA).

Table 1
Oxidation of β-CD by the TEMPO–NaBr–NaClO system [a]

Entry	TEMPO [b] ($\times 10^{-3}$)	NaBr [b]	NaClO [b]	Yield [c] (%)				
				F1	F2	F3	F4	ΣF2+F3+F4
1	25	0.4	1.3	6	11	18	25	54
2	25	0.6	1.3	6	16	32	19	67
3	25	0.8	1.3	6	15	28	27	70
4	26	0.8	1.4	10	19	26	27	72
5	25	0.8	1.6	4	20	31	39	90
6	22	0.5	1.0	11	26	26	22	74
7	22	0.7	1.0	6	21	22	17	60

[a] At 2 °C and constant pH 10.

[b] In mmol per mmol of primary alcohol.

[c] The yield was calculated with the molecular weight of the oxidized compound as the sodium salt in the case of F1, F2 and F3 fractions. Concerning fraction F4, an average molecular weight of 1279 g mol^{-1} corresponding to an oxidation degree of 4 was taken.

The samples were dissolved in a glycerol matrice and submitted to Xe (9 kV) bombardment. The molecular weights were determined by carrying out FABMS directly on the underivatized compounds.

Sample.—β-CD was purchased from Aldrich-Europe, Janssen Pharmaceutica (Beerse, Belgium). TEMPO was obtained from Fluka, (Buchs, Switzerland). All other chemicals were of commercial analytical grade.

Oxidation procedure.—The β-CD (1.134 g, 1 mmol), TEMPO (20 mg, 0.13 mmol) and NaBr (0.50 g, 4.9 mmol) were dissolved in distilled water (300 mL). Sodium hypochlorite soln (13.7% (w/v), 2.86 mL, 5.16 mmol), with the pH adjusted to 10 by addition of 4 M aq HCl, was added several times (0.4 mL at once every 2–3 min) to the soln. The pH was controlled by a pH-controller and maintained at 10 during the course of the reaction by continuously adding a 0.5 M NaOH soln. The temperature of the reaction was maintained around 2 °C during the course of the oxidation reaction. The reaction mixture was neutralized to pH 7 with a 0.05 M HCl soln, partially concd at < 50 °C under reduced pressure. The substrate was fractionated on a biogel P6 column eluted with a 0.1 M $NaNO_3$ soln. Four fractions were collected: F1, which contained the unreacted β-CD, F2, which contained the monocarboxy-β-CD (**1**), F3, which contained the three dicarboxy-β-CDs (**2**–**4**) and F4, which corresponded to a mixture of tri, tetra,…carboxy-β-CDs. The fractions were then desalted by ultrafiltration with a membrane having a molecular weight cut-off of 500 Da and freeze-dried. The several oxidation experiments were performed at 2 °C with different amounts of NaClO. The data reported in Table 1 shows that with increasing quantities of NaClO, the amount of unreacted β-CD decreased and the amount of oxidized compounds increased.

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