Cleavage of the glycosidic bonds in methyl β-D-cellotrioside in alkaline medium

I. ŠIMKOVIC, A. EBRINGEROVÁ, J. KÖNIGSTEIN, V. MIHÁLOV, and F. JANEČEK

Institute of Chemistry, Centre for Chemical Research, Slovak Academy of Sciences, CS-842 38 Bratislava

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Kinetics of alkaline hydrolysis of methyl β-D-glucopyranoside, methyl β-D-cellobioside, and methyl β-D-cellotrioside was studied using spectrophotometry and the combination of gas chromatography with mass spectrometry (GC—MS). The results of the kinetic measurements suggest that stability towards alkaline hydrolysis of the glycosidic bonds in methyl β-D-cellotrioside decreases from the model glycosidic bond to the true ones.

Йзучалась кинетика щелочного гидролиза метил-β-D-глюкопиранозида, метил-β-D-целлобиозида и метил-β-D-целлотриозида с применением спектрофотометрических методов и комбинации газовой хроматографии с масс-спектрометрией (ГХ—МС). Результаты кинетических измерений позволяют предположить, что устойчивость при щелочном гидролизе гликозидных связей в метил-β-D-целлотриозиде уменьшается при переходе от модельной гликозидной связи к реальной.

In relation to the losses on polysaccharides during the alkaline pulping processes, much attention [1—7] has been paid to alkaline degradation of glycosidic bonds in saccharides. The so far obtained results on the mechanism of glycosidic bond hydrolysis indicate the formation of anhydrosaccharides which are further converted to the degradation products.

The aim of this investigation was to contribute to the knowledge on kinetics of alkaline cleavage of glycosidic bonds employing methyl β-D-glucopyranoside (*I*), methyl β-D-cellobioside (*II*), and methyl β-D-cellotrioside (*III*) as model compounds.

Experimental

Chemicals

Methyl β-D-glucopyranoside, methyl β-D-cellobioside, and methyl β-D-cellotrioside were prepared by known procedures [8—10]. The solutions of the models used and of sodium hydroxide were prepared from freshly redistilled water and stored under nitrogen atmosphere in a refrigerator.

Kinetic measurements

One cm^3 portions of the solution, each containing 1 mg of the substrate in sodium hydroxide solution ($c(NaOH) = 0.1$—8 mol dm^{-3}), were pipetted into glass ampoules. Then the ampoules were rinsed with a stream of nitrogen, stoppered, and sealed. The samples were conditioned in silicone oil bath at temperatures within the range of 150—190 °C (± 1 °C). After the certain time intervals, the samples were taken out of the bath and cooled to ambient temperature with tap water. After opening, the volume was filled to 25 cm^3. The saccharides were determined by phenol—sulfuric acid method [11] using a spectrophotometer Spekol 10 (Zeiss, Jena) at the wavelength of 490 nm. Each of the samples was filtered before the analysis through a bacteriological filter (Rasotherm, Pore 1.6, GDR). The starting value of absorbance (A_0) was obtained so that the ampoule was immediately after sealing reopened without conditioning in the oil bath and diluted to 25 cm^3 volume. The value of absorbance after time t was designated as A_t. The rate constants were calculated from the plots $\ln A_0/A_t = f_1(t)$ by the linear regression method. Analogously, the activation energies were calculated based on Arrhenius' plots $\ln k = f_2(1/T)$, the standard calculating programs in AOS [12, 13] were modified employing the programmable calculators TI-59 and TI-58 C connected to a printer PC-100 B (Texas Instruments).

GC—MS analyses

After the saccharide determinations, the samples were adjusted to pH 8 with a Dowex 50WX8 exchange resin in H-cycle. After removal of the ion exchanger, they were concentrated to dryness *in vacuo*. *O*-Trimethylsilylation was performed in the usual manner [14]. The gas-chromatographic separations were carried out on a Hewlett—Packard 5700 A instrument using stainless steel columns packed with OV-17 ($w = 3$ %) on Chromosorb WAW DMSCS (0.177/0.149 mm). The column size was 2 m × 2.1 mm. After an initial isothermic separation (8 min), a linear temperature programming from 140 to 300 °C was applied with a heating rate of 2 °C/min. GC—MS analyses were performed on JGC-20 K and JMS-D 100 instruments. The mass spectra ($E = 22$ eV) were recorded at an electron emission of 300 μA in an inert atmosphere of helium.

GC—MS data for the *O*-trimethylsilyl derivatives:

I: retention time 6 min.

II: relative retention time 2.13.

III: relative retention time 3.03.

IV: relative retention time 1.43. MS (m/z (I_{rel}/%)): 451(17), 422(5), 361(9), 333(15), 332(28), 319(59), 307(60), 244(31), 217(100), 205(25), 189(39), 147(25), 129(67), 103(43), 73(29).

V: relative retention time 0.25. MS (m/z (I_{rel}/%)): 365(7), 333(14), 332(9), 323(5), 295(5), 291(14), 290(6), 263(14), 189(6), 175(5), 149(16), 147(25), 133(3), 115(27), 75(100), 73(76).

VI: relative retention time 0.3 MS (m/z (I_{rel}/%)): 369(0.3), 334(0.5), 319(1.4), 311(1.4), 295(0.7), 282(0.5), 223(1), 221(2), 207(4), 205(2), 171(2), 170(14), 163(2), 149(11), 148(18), 147(75), 143(4), 135(1), 133(4), 131(5), 121(3), 120(26), 117(3), 116(4), 105(20), 89(18), 77(64), 75(100), 73(39).

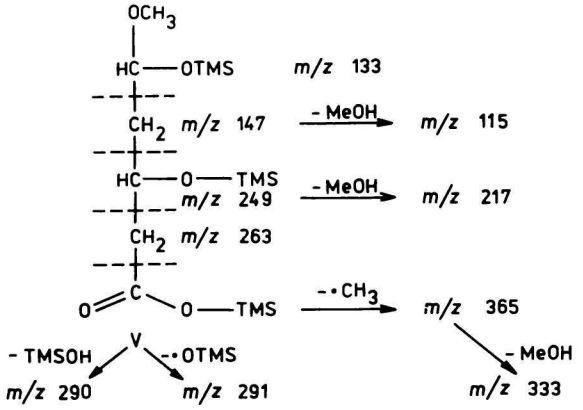
VII: relative retention time 0.38. MS (m/z (I_{rel}/%)): 393(0.6), 369(5), 319(1.5), 282(22), 207(4), 205(2), 170(8), 147(91), 120(10), 75(100), 73(45).

Results and discussion

The GC and GC—MS analyses showed that, in case of *II* and *III*, methyl β-D-glucopyranoside (*I*) could be detected in the initial stage of alkaline degradation as a result of alkaline hydrolysis of the glycosidic bonds. Besides, further four substances (*IV*, *V*, *VI*, and *VII*), which have not yet been described in the literature, were detected. They belong to the minor degradation products as their concentrations were at least by two orders lower than that of *I*. Presumably, they were formed from *I* since they appeared in each of the alkaline degradation mixtures resulting from the three model compounds used. This was concluded on the basis of GC analyses. No other degradation products, nor 1,6-anhydro-β-D-glucopyranose, were identified under these conditions.

Taking into consideration fragmentation paths of similar substances [15], we assigned to the compound *V* the structure shown in Scheme 1. Unfortunately, we were unsuccessful in attempt to identify the other compounds *IV*, *VI*, and *VII*. It is a shortcoming of *O*-trimethylsilyl derivatives that their mass spectral fragmentation often provides too little information on the structure of unknown compounds. The same problem was encountered also by other authors [16] examining *O*-trimethylsilyl derivatives of the alkaline degradation products of saccharides.

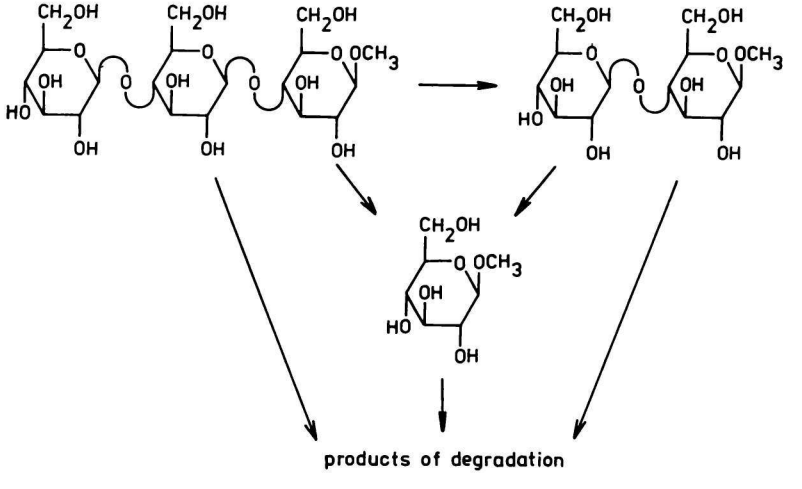
The formation of the compound *V* indicates that the reaction of pyranose-ring opening can take place without rupture of the methyl glycosidic bond in methyl β-D-glucopyranoside. This is in agreement with the report on isolation of acetal structures during the study of alkaline-oxygen degradation of methyl β-D-glucopyranoside using GC—MS analysis of their *O*-trimethylsilyl derivatives [17].



Scheme 1

Based on our results, we propose for alkaline degradation of *III* the reaction pathway shown in Scheme 2.

In general, the phenol—sulfuric acid method enables to determine all the saccharides built of more than four carbon atoms with only some rare exceptions, *e.g.* aldonic and saccharinic acids [18]. Thus, 1,6-anhydro-β-D-glucopyranose as well as any of the polysaccharides are amenable to this method. Deoxysaccharides are slightly susceptible to this analysis [11]. The differences in response to the



Scheme 2

detection of the model compounds studied herein can be neglected and considered proportional to the concentration of starting compound (in case of the model *I*) and to the overall concentrations of saccharides in case of the models *II* and *III*.

When following the decrease in the substrate concentration as a function of time (1 M-NaOH, the range of temperatures 150—190 °C, inert atmosphere of nitrogen) using phenol—sulfuric acid method, the rate constants corresponding to a first-order reaction were obtained (Table 1). The plots of rate constants *vs.* concentration of NaOH are curves which at higher values of NaOH concentration are almost parallel with the axis of abscissas. This shape excludes the possibility of a direct participation of OH anion in the rate-controlling reaction step. The calculated activation energy (E_A) for alkaline degradation of *I* under the above-mentioned conditions was 169.9 kJ mol^{-1}.

Table 1

Values of the rate constants k^I/h^{-1} for alkaline degradation of *I* (0.1—8 M-NaOH, 150—190 °C, nitrogen atmosphere)

θ/°C	c(NaOH)/(mol dm^{-3})			
	0.1	1	3	8
150	—	0.0019	—	—
160	—	0.0057	—	—
170	0.0019	0.0166	0.0259	0.0445
180	0.0033	0.0458	0.1169	0.1712
190	—	0.1212	—	—

When examining the alkaline degradation of the models *II* and *III*, in addition to the starting compounds, we could detect in the initial stage also *I* and/or *II*, which resulted from hydrolysis of the glycosidic bonds. This made impossible to determine the hydrolysis rate constants for the individual glycosidic bonds, but only the experimental rate constants k^{II} and k^{III} (Tables 2 and 3). The values of the rate constants are close to that of k^I for degradation of *I* so confirming that the degradation of the model *I* is the slowest, rate-determining step. A comparison of the activation energies for alkaline degradation of the model *II* (165.7 kJ mol^{-1}) and *III* (142.6 kJ mol^{-1}), calculated from the rate constants k^{II} and k^{III} (1 M-NaOH, 150—190 °C, nitrogen atmosphere) with that of degradation of model *I* (169.9 kJ mol^{-1}) shows that the stability of the glycosidic bonds in the model *III* towards hydrolysis in alkaline medium decreases from the model bond to the true ones.

When comparing the alkaline degradation rate constants obtained in our study with those of alkaline degradation of reducing saccharides during peeling-off

Table 2

Values of the experimental rate constants k^{II}/h^{-1} for alkaline degradation of *II* (0.1—8 M-NaOH, 150—190 °C, nitrogen atmosphere)

θ/°C	c (NaOH)/(mol dm^{-3})			
	0.1	1	3	8
150	—	0.0016	—	—
160	—	0.0037	—	—
170	0.0041	0.0134	0.0178	0.0574
180	0.0056	0.0332	0.0377	0.0647
190	—	0.0857	—	—

Table 3

Values of the experimental rate constants k^{III}/h^{-1} for alkaline degradation of *III* (0.1—8 M-NaOH, 150—190 °C, nitrogen atmosphere)

θ/°C	c (NaOH)/(mol dm^{-3})			
	0.1	1	3	8
150	—	0.0023	—	—
160	—	0.0050	—	—
170	0.0062	0.0143	0.0422	0.1245
180	0.0091	0.0318	0.1721	0.2291
190	—	0.0719	—	—

reaction [19, 20], it is evident that alkaline hydrolysis of glycosidic bonds is many times slower and occurs at far higher temperatures. These conclusions are in agreement with the knowledge on the alkaline delignifications. They suggest that stabilization towards alkaline hydrolysis of the cellulose macromolecule by modification of its reducing end moieties has only a limited effect [21]. In fact, this modification cannot prevent the cleavage of glycosidic bond *via* alkaline hydrolysis which produces new reducing end groups liable to the peeling-off reaction. Furthermore, alkaline hydrolysis significantly reduces the average polymerization degree and produces low-molecular alkali-soluble saccharide fractions. In consequence, this has a detrimental effect on the yields and the physicochemical properties of pulps.

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