

PREPARATION OF MACRO CROWN ETHER-LIKE COMPOUNDS BY
THE SMITH DEGRADATION OF CYCLODEXTRINS

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The ability of cyclodextrins to give inclusion or "host-guest" complexes by insertion of various organic and inorganic molecules into their hydrophobic cavity is well-known. But these cavities are remarkably rigid. We have produced a crown ether-like compound starting from beta-cyclodextrin, in which the inner cavity is enlarged and more flexible.

Beta-CD was subjected to Smith-degradation involving the following steps: periodate oxidation, reduction and derivatization.

The polyaldehyde obtained by complete periodate oxidation of beta-CD was reduced with sodium borohydride to the corresponding polyalcohol.

The crown ether-like macro-cycle obtained by the above process was subjected to several chemical modifications. By methylation we have obtained compounds possessing solubility both in water and in organic solvents and ability to form complexes. On the other hand, by tosylation and LiAlH_4 reduction of the polyalcohol we managed to get a "macro cyclic ring" of completely apolar character. The present study deals with the structural investigation of the above compounds. The so-called J-ECHO ^{13}C -n.m.r. spectra of the acetylated and methylated derivatives show total symmetry of the compounds.

The establishment of a similar reaction sequence with both alfa- and beta-cyclodextrin is in progress

Introduction

There are two general methods for the determination of the character of the linkage of polysaccharides: periodic acid oxidation (1,2) and methylation analysis (3). The present paper gives details of the possibilities for the utilization of the periodate oxidation.

The most widely applied method for the transformation of the polyaldehydes furnished by the above oxidation process, is the Smith-degradation involving successive oxidation with periodate in aqueous medium, removal of the iodate and periodate ions and subsequent reduction with sodium borohydride. The resulting polyalcohol is then hydrolyzed and the products in the hydrolysate are analyzed. Of the polysaccharides amylopectin (4) and cellulose (5) have been first analyzed by this method as demonstration of the usefulness of the procedure which has emerged recently as a generally employed way of analysis of polysaccharides.

The modified Smith-degradation, completed with methylation, is also a powerful method for the examination of polysaccharides. The general applicability of this process and its advantage over both the classical methylation-analysis and the conventional Smith degradation has been demonstrated by Nánási and Lipták (6,7).

The present paper deals with a novel possibility of application of the Smith-degradation.

Beta-cyclodextrin was converted by Smith-degradation into crown ether-like derivatives. Cyclodextrins are macrocyclic carbohydrate molecules built from 6, 7 or 8 monomeric glucose units and arranged in a torus which are denoted alfa, beta or gamma-cyclodextrins, respectively. Such connection of the glucose moieties in the cyclodextrins produces a rigid and conical molecular structure with a specific inner cavity (8).

The phenomenon of inclusion complexation between cyclodextrins and a variety of organic and inorganic molecules has prompted research in various disciplines. Biochemists have found that the inclusion process is analogous to the binding of enzymes and substrates, while pharmacologists have used cyclodextrins to stabilize drugs and render them more compatible with body fluids (9).

Our aim was to produce crown ether-like compounds from cyclodextrins, in which the inner cavity is enlarged and more flexible. By this way we wanted to obtain possibilities for complexation of large molecules, as well.

The cleavage of the ring of beta-cyclodextrin was accomplished by periodate oxidation. The kinetics of the oxidation of cyclodextrins with periodate have been studied by French et al. (10). During our work the Smith-degradation process was somewhat modified as the polyalcohol, obtained after the reduction, was converted into acetylated-, methylated and tosylated analogues.

Materials and methods

Beta-cyclodextrin is a product of the Chinoin Pharmaceutical Works (Budapest, Hungary) and its purity was

min. 99% (after drying to constant weight). No other carbohydrate component could be detected by t.l.c. or h.p.l.c. Reactions were monitored by t.l.c. on Kieselgel 60 F 254 (Merck), detection by charring with sulfuric acid. Kieselgel G (Reanal) was used for short-column chromatography. Optical rotations were measured with a Perkin-Elmer 241 automatic polarimeter. I.R. spectra were recorded with a Perkin-Elmer 700 instrument. ^{13}C -n.m.r. spectra (for solution in CDCl_3) were taken at room temperature with a Bruker WP-200 SY spectrometer, using CDCl_3 as an internal reference. G.l.c. was performed at 80°C with a Hewlett-Packard 5840 A instrument, and a column (1.2 m x 2 mm) of 10% UCW 982 on Gas Chrom-Q (80-100 mesh) with N_2 as carrier gas at 20 ml/min.

Beta-CD-polyaldehyde

To a solution of beta-cyclodextrin (11.34 g) in about 450 ml of water a solution of NaIO_4 (17.9 g; 8.4 M) in 500 ml of water was gradually added. The mixture was diluted to 1 l. with water and it was kept in the dark at 40°C . For the determination of the periodate consumption an aliquot of this solution was diluted 100 times and the absorption was measured with a Beckmann DB spectrophotometer at 222.5 nm.

The oxidation was completed within 96 hr ; periodate consumption: 1.03 mol. Ethyleneglycol (10 ml) was added to the reaction mixture, it was evaporated to dryness and after removal of the inorganic salts beta-CD-polyaldehyde was isolated by evaporation. T.l.c. and g.l.c. showed that no unoxidized glucose was present in the 2N sulfuric acid hydrolysate of the polyaldehyde.

Beta-CD-polyalcohol

A solution of the polyaldehyde (11 g) in water (500 ml) was treated with NaBH_4 (5.29; 14 M) at room temperature for 24 h. After neutralization with conc. acetic acid the mixture was evaporated. Coevaporation with methanol (5x30 ml) afforded 9.83 g of the syrupy polyalcohol.

Beta-CD-polyacetate

The beta-CD-polyalcohol (4 g) was acetylated with acetic anhydride (40 ml) in abs. pyridine (40 ml) by stirring overnight at room temperature and then by warming up to 60°C . The reaction mixture was poured onto ice-water, the precipitated beta-CD-crown was filtered off and dissolved in dichloromethane. The organic layer was washed with 0.5 N H_2SO_4 and then with water until neutral, dried over Na_2SO_4 and evaporated. Purification with column chromatography (Silicagel, 8:2 dichloromethane-acetone) gave 3.5 g of foam-like material. $\alpha_D = -0.1$ (chloroform). ^{13}C -n.m.r. data: δ 170.2 (COCH_3), 100.2 (C-1), 75.6 (C-4.5),

64.2 (C-2), 63.4 (C-3.6), 20.3 (CO-CH₃)

Beta-CD-polymethylether

3.5 g acetylated beta-CD-crown was dissolved in abs. methanol (20 ml) and the pH of the solution was adjusted to 9.0 by the addition of solid NaOCH₃. After standing overnight the mixture was neutralized with Amberlite IR 120 resin, it was filtered and the filtrate evaporated.

The dried deacetylated product (0.9 g) was dissolved in DMF (15 ml) and 1.2 g NaH (3 fold excess/OH groups) was added. After stirring for 20 min. a six-fold excess of methyl iodide (6.23 ml) was added, the excess of NaH was decomposed with methanol and the mixture was diluted with dichloromethane. The salts were filtered off and the filtrate was washed several times with water. After drying over Na₂SO₄ the solvent was evaporated and the product was purified on a Silicagel column (95:5 dichloromethane-methanol) (0.8 g syrupy). The degree of methylation was also checked with g.l.c. examination. (The NaBH₄ reduction of the hydrolysate of the product with TFAA resulted in the formation of dimethylerythritol and ethyleneglycol monomethylether and this latter can be detected by g.l.c.) ¹³C-n.m.r. data: δ 101.4 (C-1), 76.3 (C-4.5), 58.7 (OMe), 74.2 (C-2), 70.1 (C-3.6).

Beta-CD-polytosylate

The polyalcohol (1 g), purified by acetylation and subsequent deacetylation, was tosylated in abs. pyridine (20 ml) by the addition of TSOCl (1.5 mol/OH group). The reaction was monitored by t.l.c. The mixture was poured onto ice-water and washed up in the usual manner. The product was purified by chromatography on a Silicagel column with 8:2 toluene-acetone as the eluent.

The tosylated product (4 g) was reduced with LiAlH₄ into a totally apolar crown ether-like compound: a solution of the tosylate in 1:1 benzene-ether was treated with LiAlH₄ (0.89 g) at reflux temperature for 48 h. After the starting material had disappeared (t.l.c.) the mixture was cooled, diluted with ether, the excess of the reagent was decomposed with ethyl acetate and the produced aluminium hydroxide was precipitated with water. The precipitate was decanted, washed several times with water the combined organic layer was washed, dried and concentrated (0.5 g syrupy).

Results and discussions

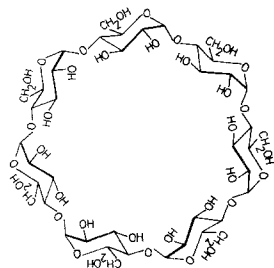


Fig. 1: β -CD

The cleavage of the ring of beta-cyclodextrin (Fig. 1) with periodate proceeds by splitting of the bond between the C-2 and C-3 atoms of each glucose unit connected with 1-4 linkages, no formaldehyde or formic acid is produced during the oxidation and a periodate consumption of 1 mol per glucopyranosyl moiety is measured.

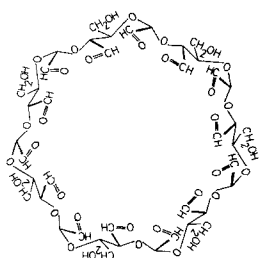


Fig. 2:
 β -CD-polyaldehyde

The oxidation of beta-cyclodextrin was performed in aqueous medium at 50C in the dark to avoid side-reactions by using 1.2 M of NaIO_4 for each glucose unit. Monitoring of the process was carried out by the direct photometric measurement of the consumed metaperiodate at 222.5 nm (11).

After completion of the oxidation the reaction was frozen by the addition of ethyleneglycol, the inorganic salts were removed and the formed beta-CD-polyaldehyde (Fig. 2) was isolated. Completion of the reaction was stated when no glucose could be detected in the sulfuric acid hydrolysate of the oxidized beta-cyclodextrin.

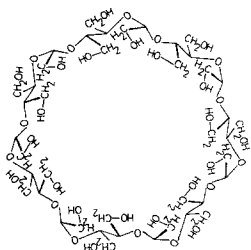
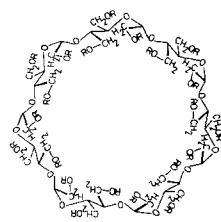


Fig. 3:
 β -CD-polyalcohol

The produced polyaldehyde was then reduced into the corresponding polyalcohol in aqueous medium by using 2 molecular equivalent of NaBH_4 for each glucose unit. After neutralization of the reaction mixture the borate esters were removed by repeated codistillation with methanol.

The isolated polyalcohol (Fig. 3) was derivatized by acetylation, methylation and tosylation (Fig. 4).



R = $\text{CH}_2\text{C}_6\text{H}_4\text{-SO}_2$
P = H
S = Ac
Q = CH_3

Fig. 4:
 β -CD-derivatives

Acetylation was performed with a 1:1 mixture of acetic anhydride and anhydrous pyridine. The acetylated beta-CD-crown was purified by column chromatography and its structure was supported by ^{13}C -n.m.r. spectroscopy. The ^{13}C -n.m.r. spectrum of the acetylated beta-CD-crown is shown on Fig. 5.

The preparation of the methylated beta-CD-crown was accomplished in one-step by Hakamori methylation (12) modified by Lindberg (13) of the pure poly-

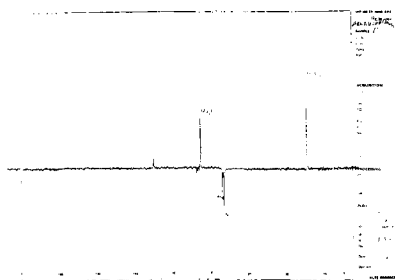


Fig. 5: ^{13}C -n.m.r. spectrum of the acetylated β -CD-crown

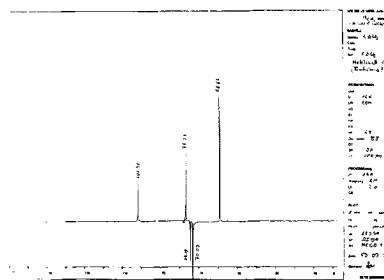


Fig. 6: ^{13}C -n.m.r. spectrum of the methylated β -CD-crown

alcohol obtained from the acetylated analogue by means of Zemplén transesterification and subsequent purification on Silicagel short-column. The degree of methylation was checked by i.r. spectroscopy and gas chromatography. The i.r. spectra of this methylated beta-CD-crown showed no absorption at 3400 cm^{-1} confirming permethylation. Upon hydrolysis of this latter compound followed by reduction with NaBH_4 only dimethylerythritol and ethyleneglycol monomethylether could be detected.

It is to be noted that the methylated beta-CD-crown ether – similarly to the methylated beta-CD-dissolves both in organic solvents and water allowing wide utilization for complexation. From methylated beta-CD-crown ether a 5-6% aqueous solution can be prepared.

The J-ECHO ^{13}C -n.m.r. spectra of the acetylated and methylated beta-CD-crown ethers were obtained with a Bruker WP 200 SY instrument (Figs 5 and 6): with the J-ECHO pulse sequence the carbons bearing two protons appear with reverse phase as compared to the mono- and tri-protonated ones. Consequently, C-1, C-4 and C-5 give peaks of positive intensity whereas the signals of C-2, C-3 and C-6 are of negative intensity. The J-ECHO spectra of the acetylated and methylated beta-CD-crown ethers showed a complete symmetry of these molecules being optically inactive due to the symmetric substitution pattern of the meso-erythritol units.

Tosylation of beta-CD-crown ether was carried out with tosyl chloride in pyridine. Reduction of the produced tosylate with LiAlH_4 afforded a completely apolar crown ether-like compound the structure of which is being investigated (Fig. 7). Studies in similar reaction sequence with alfa- and gamma-cyclodextrins is now in progress.

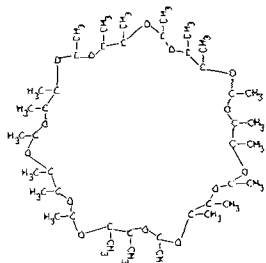


Fig. 7: Macro crown ether like compound from β -CD

References

1. Bouveng, H.O., Lindberg, B., Advan. Carbohydr. Chem., (1960), 15, 87
2. Hay, G.W., Lewis, B.A., Smith, F., Methods Carbohydr. Chem., (1965) 5, 377
3. Hirst, E.L., Percival, E., Methods Carbohydr. Chem., (1965) 5, 287
4. Goldstein, I.J., Hamiltin, J.K., Smith, F., J. Am. Chem. Soc., (1959) 81, 6252
5. Goldstein, I.J., Hamiltin, J.K., Huffman, G.W., Smith, F., J. Org. Chem., (1962) 27, 3962
6. Nánási, P., Lipták, A., Carbohydr. Res., (1973) 29, 193-199
7. Nánási, P., Lipták, A., Carbohydr. Res., (1973) 29, 201-207
8. Szejtli, J., Cyclodextrins and their Inclusion Complexes (Akadémiai Kiadó, Budapest, 1982)
9. Szejtli, J., Starch, (1981) 23, 387
10. French, D., McIntire, R.L., J. Am. Chem. Soc., (1950) 72, 5148-5150
11. Aspinall, G.O., Ferrier, R.J., Chem. and Ind. (London) (1957) 1216
12. Hakomori, S., J. Biochem. (Tokyo), (1964) 55, 205-208
13. Lindberg, B., Methods Enzymol., (1972) 28, 178-195