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REGIO- AND STEREO-SELECTIVE DEUTERIUM LABELLING OF β–CYCLODEXTRIN.

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SUMMARY

Reliable methods for the selective deuteration of β-cyclodextrin are described. Kinetic studies of the proton-deuterium exchange catalysed by Raney nickel in deuterium oxide show dramatically different behaviours of positions 2, 6 and 3 and allow selective labelling. Deuteration of protons at position 2 can be achieved by performing the exchange procedure at room temperature or preferably at 310 K. Under these conditions, no other positions are affected. At refluxing temperature, protons of the methylene group rapidly exchange and, at longer reaction times, deuteration at C3 takes place. A combination of direct and back exchange cycles affords cyclodextrins labelled on any of the sensitive positions. "Organic shift reagents" are used to assist the NMR analysis of labelled samples and have shown some stereoselectivity for deuteration of the C6 methylene group protons.

Keywords : Cyclodextrins, NMR, Deuterium labelling, Stereoselectivity.

INTRODUCTION

Cyclodextrins are cyclic oligosaccharides consisting of 6, 7 or 8 D-glucopyranose units (α , β , and γ -cyclodextrins, respectively) linked by $\alpha 1 \rightarrow 4$ bonds (1). Their relatively hydrophobic internal cavity can accomodate various organic molecules as guests leading to improved solubility in aqueous media and protection against degradation processes such as

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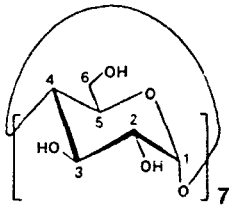
oxidation. Inclusion complexes have been described for a wide variety of organic molecules and a fast growing number of applications in the industrial and pharmaceutical fields are reported (2). β-cyclodextrin is by far the most widely used compound due to the optimal size of its internal cavity (ca. 8 Å) and will only be considered throughout in this paper.

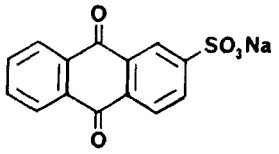
Besides their use as carriers, cyclodextrin derivatives bearing long aliphatic chains have been recently described as a new class of amphiphilic molecules which can readily be incorporated into lipid bilayers and can also exhibit lyotropic behaviour as pure compounds leading to organized phases (vesicules, lamellar systems ...) in selected solvents (3,4). Our interest in this field led us to consider labelling of cyclodextrins with deuterium since ^{2}H NMR is known as a valuable tool to investigate molecular order and dynamics in model membrane systems (5). The use of deuterium as a spectroscopic probe requires however specific labelling for a comprehensive analysis of the experimental data. A general procedure for deuteration of carbohydrates has been presented (6,7) involving exchange in deuterium oxide catalysed by Raney nickel. The complete deuteration of α-cyclodextrin (8) and β-cyclodextrin (9) at positions 2, 6 and 3 has been described but no attempts were made to achieve regioselective labelling. We wish to show here that, taking into account the highly different sensitivities of these protons towards exchange, labelling at given sites is possible. Furthermore, as the ^{1}H NMR spectra used to identify the deuterated positions remain poorly resolved, even at very high magnetic fields, newly developped procedures involving organic shift reagents (10) will be used to derive a very precise determination of the degree of exchange of all protons.

RESULTS AND DISCUSSION

The 500 MHz ^{1}H NMR spectra of β-cyclodextrin in ^2H_2O are presented in Figure 1 in the absence and in the presence of the shift reagent ASANA showing that inclusion of this aromatic compound leads to a completely resolved spectrum. These spectra will be used as references throughout and specific labelling of the available positions will be considered successively. It is worth noting that, in the presence of the ASANA shift reagent, H6 (pro-R) and H6' (pro-S) protons, as assigned in ref. (11), appear in reverse order, relative to the normal spectrum (10).



β-cyclodextrin



Anthraquinone
2-sulfonic acid,
Sodium salt
(ASANA)

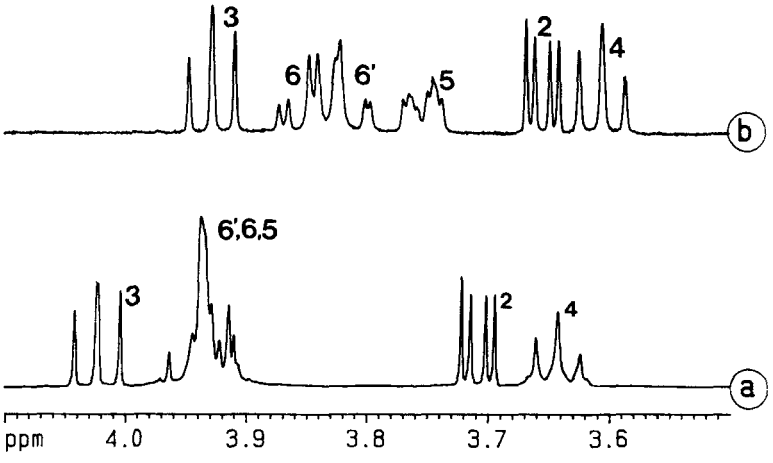


Figure 1.

Partial 500 MHz ^{1}H NMR spectra of β-cyclodextrin (5 mM) at 298 K in the absence (a) and in the presence (b) of 10mM ASANA.

Selective deuteration of β-cyclodextrin at C2 : 2 $\{^2H\}_7$ β-cyclodextrin.

Preliminary experiments have shown that protons at C2 position exchanged extremely rapidly at reflux temperature. Selectivity could then be achieved using milder conditions where no other protons were affected. It was found that at 310 K, complete (97%) exchange of protons at C2 was obtained within 24 hours. At room temperature, ca. 6 days are required to obtain similar results. This is shown in Figure 2a where signals from H2 are completely absent and those from coupled spins are simplified as expected, H1 and H3 being indeed converted into a singlet and a doublet, respectively. The spectrum obtained in the presence of ASANA shows that other strongly coupled signals remain unaffected .

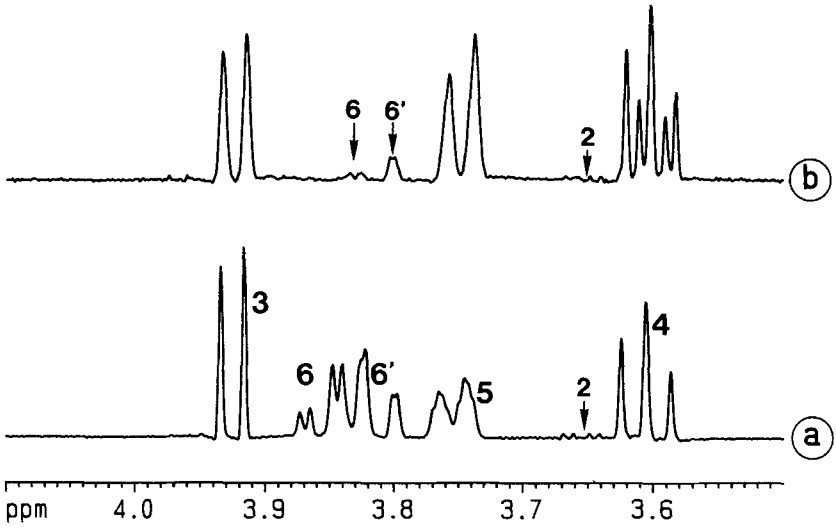


Figure 2

Partial 500 MHz proton NMR spectra of β-cyclodextrins deuterated at C2 (a), C2,C6 and C3 (30%) (b) in the presence of the ASANA shift reagent.

Deuteration at C2 and C6 positions : 2,6,6' $\{^2H\}_{21}$ β-cyclodextrin.

When the catalytic exchange reaction is allowed to proceed at higher temperatures (refluxing conditions), other positions can be deuterated. A complete kinetic study was performed by withdrawing aliquots of the reaction mixture and monitoring the deuteration level by ^1H NMR. Figure 3 shows the behaviour of H2, H6, H6' and H3 protons.

As already mentionned, H2 protons disappear extremely rapidly under the present conditions. H6 and H6' protons (proR and proS respectively) exhibit slightly different exchange rates. This can only be seen in the presence of the ASANA shift reagent since completely resolved spectra are obtained. Figure 2b shows a situation where 84 and 96% of H6' and H6 protons have been exchanged, respectively. Intermediate situations lead to complicated spectra due to isotope effects and unresolved H-D couplings. A similar stereoselectivity towards deuterium exchange has been evidenced for methyl α-D-glucopyranoside (12).

Although the stereoselectivity remains poor, the possible differential labelling of the two methylene protons is expected to be of high value for a correct assignment of deuterium NMR signals in condensed phases. The absence of any modification in the coupling constants of the

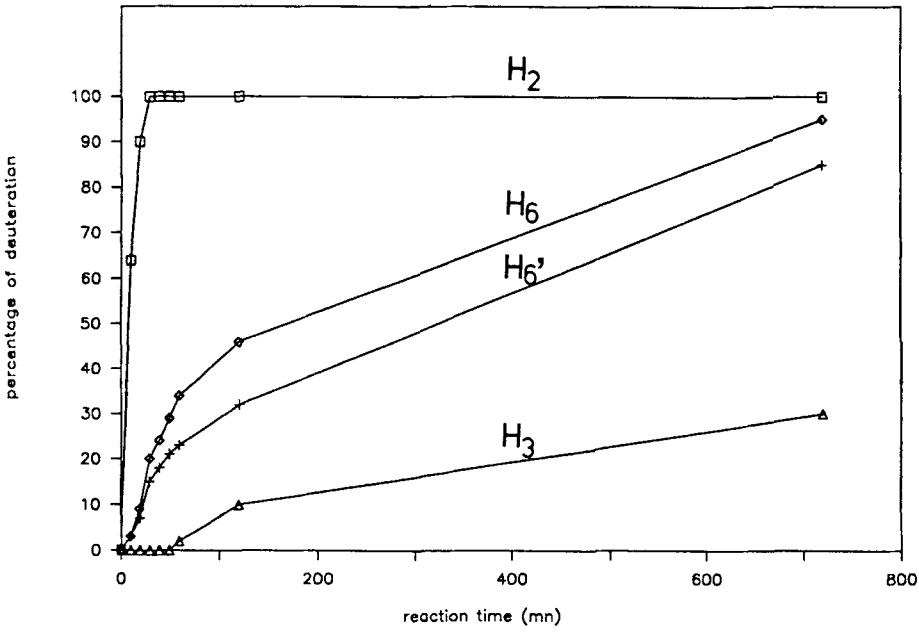


Figure 3
Exchange kinetics of H2, H6, H6' and H3 protons of β-cyclodextrin at 375 K

unmodified signals proves that no epimerisation process takes place under the present experimental conditions, even at long times. Some degradation is hovewer observed after 48 h exchange at refluxing temperature.

Deuteration at C3 position : 2,6,6',3 $\{^2H\}_{28}$ β-cyclodextrin.

It has been reported in Figure 3 that at long reaction times, H3 protons slowly exchange to deuterons. If the reaction is allowed to proceed for 12h under reflux, a 30% deuteration at C3 is obtained. As expected, part of the triplet line from H4 is converted into a doublet (Fig. 2b). After 48h, ca. 80% deuteration could be obtained but a slight chemical degradation was also obvious.

Back-exchange experiments.

As the reactivities of C2, C6 and C3 strongly differ, specific deuteration on any among the three avalaible positions can be achieved by controlled successive deuteration and back-exchange in H_2O. This procedure has been successfully used to afford β-cyclodextrin labelled at C6 or C3 selectively by controlled back-exchange.

CONCLUSION

The methods presented here for regioselective deuteration of β-cyclodextrin make use of cheap reagents and mild conditions and can be scaled up to large amounts of starting material suitable for the preparation of substituted derivatives. Proton NMR in the presence of organic shift reagents provides a very fast and efficient method for checking the position and extent of deuteration. Further work in this field involves the study of the two other cyclodextrins and preliminary results indicate very different behaviours towards exchange in these conditions relative to β-cyclodextrin. Finally, this procedure is being currently extended to labelling with tritium and the results will be presented in a very near future.

EXPERIMENTAL

β-cyclodextrin (Roquette, France) was freeze-dried twice from ^2H_2O (CEA, France) to exchange labile hydroxyl protons. Raney nickel (Aldrich) was washed four times with deuterium oxide by decantation. A reaction mixture containing β-cyclodextrin (200 mg) in deuterium oxide (30 mL) and Raney nickel (2 mL, settled volume) was gently stirred under a nitrogen atmosphere in a temperature-controlled silicone oil bath. For kinetic experiments, aliquots (1 mL) were withdrawn, cooled rapidly and filtered on polycarbonate filters (Millex) in the presence of a small amount of Chelex 100 chelating resin (Bio-Rad). The NMR spectra were run immediately and the solution freeze-dried for further use. The bulk solution was processed in the same way. Sodium anthraquinone 2-sulfonate (ASANA) was obtained from Fluka. NMR experiments were performed at 500 or 200 MHz using Bruker WM500 and AC200 spectrometers, respectively. Chemical shifts are reported relative to external tetramethylsilane. All spectra were collected using 5 sec. relaxation delay and 45° flip angles to ensure reliable determination of the integrals.

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