Pergamon

Tetrahedron Letters, Vol. 35, No. 31, pp. 5591-5594, 1994
Elsevier Science Ltd
Printed in Great Britain
0040-4039/94 $7.00+0.00

0040-4039(94)01150-8

C-Sucrose vs. *O*-Sucrose: Different Conformational Behavior in Methanol Solutions Containing Ca^{2+}

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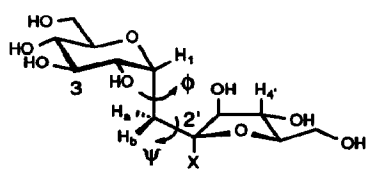
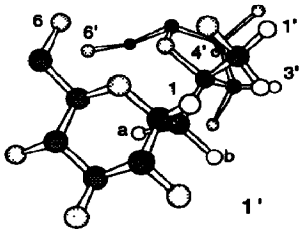
Abstract: NMR chemical shift, $^3J_{HH}$, $^3J_{CH}$, nuclear Overhauser enhancement, and optical rotation data suggest that *C*-sucrose undergoes a conformational change in methanol containing Ca^{2+}, whereas the parent *O*-sucrose does not.

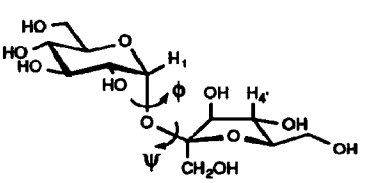
Comparing the conformational behavior of *C*-glycosides with naturally occurring *O*-glycosides has been a topic of ongoing interest in these laboratories.[1] During conformational studies[2] of *C*-sucrose (**1**), we discovered that a cation-induced conformational reorganization takes place in dilute methanolic solutions of certain cations (Ca^{2+}, Sr^{2+}, and Ba^{2+}). This is interesting, because *O*-sucrose (**2**) does not complex cations to any appreciable extent in either methanol or aqueous[3] solution.



1: X = CH_2OH
1-^{13}C: X = $^{13}CH_2OH$



1'



2

Addition of 3 eq of Ca^{2+} to a 10 mM solution of *O*-sucrose (**2**) in CD_3OD imparts virtually no effect upon its ^1H NMR spectrum (Figure 1). Small shifts are observed for **2** after the addition of greater than 10 eq Ca^{2+} (data not shown). In contrast, the ^1H NMR spectrum of *C*-sucrose undergoes large changes in chemical shift[4,5] with the addition of as little as 1 eq of Ca^{2+}, with limiting chemical shifts obtained after the addition of ca. 3 eq Ca^{2+}. In particular, large downfield shifts are observed for H.1, the H.1' pair, and one of the H.6 protons. Upfield shifts were observed for the fructofuranosyl H.3' and H.4' protons, which become nearly degenerate with H.5' in the presence of Ca^{2+}. The normally degenerate H.a and H.b protons shift to yield a readily analyzed ABX spin system in the presence of added salts.

A picture of the Ca^{2+}-induced conformational changes in *C*-sucrose is revealed by the ^1H-^1H and ^1H-^{13}C spin-spin coupling constants used to monitor the glycosidic torsional angles ϕ and ψ (Figure 1). In the absence of calcium, these coupling constants are indicative of a differential degree of flexibility at the ϕ/ψ linkage in *C*-sucrose: (i) the large/small pyranosyl ^3J values suggest that the *C*-glycosidic bond at the pyranoside site preferentially adopts an exo-anomeric conformation and (ii) the nearly equivalent ψ $^3J_{CH}$ values do not reflect any particular conformational preference. Solution NOE studies are consistent with averaging at the ψ linkage.[6] Upon addition of Ca^{2+}, the glycosidic coupling constants in **1**-^{13}C indicate that a conformational reorganization has taken place. A small effect is observed at ϕ, with the exo-anomeric conformation stabilized to a more fully staggered orientation. Dramatic effects are seen at ψ, as the $^3J_{CH}$ assume values indicative of a preferred fructofuranosyl exo-anomeric conformation, similar to that observed in the crystal structure of *C*-sucrose octaacetate.[2] In addition, a $^4J_{HH}$ W-coupling can be detected between H.a and one of the H.1' protons. This long-range coupling indicates the -CH_2OH rotational degrees of freedom are reduced in the presence of calcium.

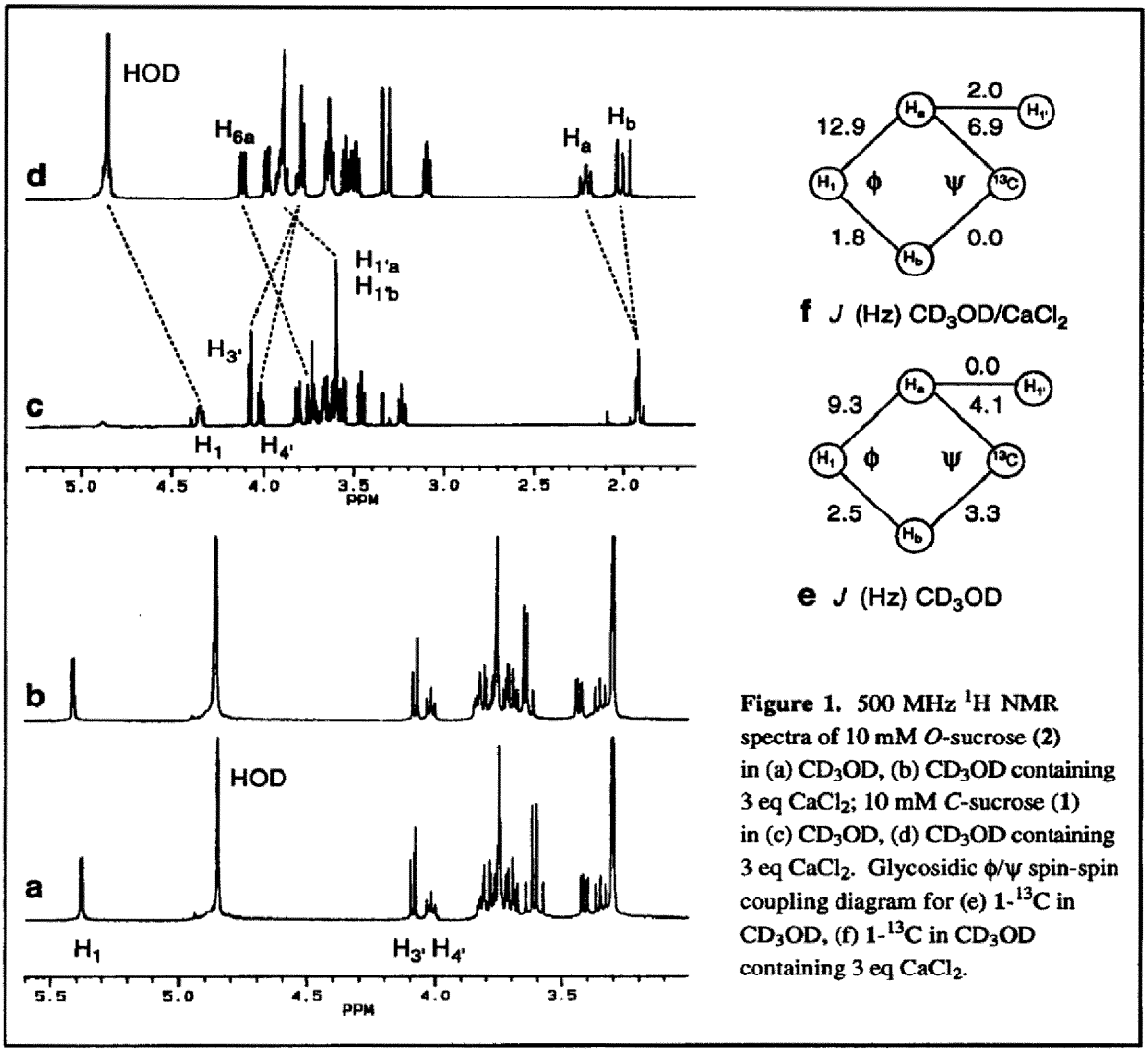


Figure 1. 500 MHz ^{1}H NMR spectra of 10 mM *O*-sucrose (**2**) in (a) CD_3OD, (b) CD_3OD containing 3 eq $CaCl_2$; 10 mM *C*-sucrose (**1**) in (c) CD_3OD, (d) CD_3OD containing 3 eq $CaCl_2$. Glycosidic ϕ/ψ spin-spin coupling diagram for (e) 1-^{13}C in CD_3OD, (f) 1-^{13}C in CD_3OD containing 3 eq $CaCl_2$.

Using these glycosidic ϕ/ψ coupling constants as a guide, it is possible to arrange the conformation of *C*-sucrose in an extended form, fixing the C.1' hydroxymethyl group in a manner to satisfy the observed W-coupling, cf. H.a-C-C-C-H.1' in Structure **1'**. This produces an oxygen-rich pocket comprised of the pyranosyl and furanosyl ring ether oxygens (O.5 and O.5'), and three hydroxyl groups (OH.1', OH.6', OH.6), i.e. Structure **1'**.

Intra-residue NOE experiments were used to further test the hypothesis of an extended conformation in the presence of Ca^{2+}. As stated earlier, the H.1-H.4' interaction in *C*-sucrose is well-documented, and is consistent with averaging at the ψ linkage.[6] We reasoned that a diminished H.1-H.4' NOE for **1** in the presence of calcium could be taken as further evidence for the extended geometry. As implied earlier, the H.3' and H.4' chemical shifts overlap in the presence of calcium, making this experiment difficult to interpret. Accordingly,

we prepared *C*-sucrose labeled with deuterium at the H.3' position (**1-*d***). As shown in Figure 2b, irradiation of H.1 in **1-*d*** in the absence of Ca^{2+} produced a 3.5% enhancement at H.4'. Upon addition of Ca^{2+}, the same irradiation produced enhancements at H.2, H.1' and H.a, with no significant enhancement observed at H.4'. Therefore, the diminished H.1-H.4' NOE, in conjunction with the calcium-induced changes in glycosidic coupling constants, suggests that calcium complexation involves both residues, and provides a conformational shift towards an extended conformation (**1'**) in which both ϕ and ψ preferentially adopt exo-anomeric conformations.

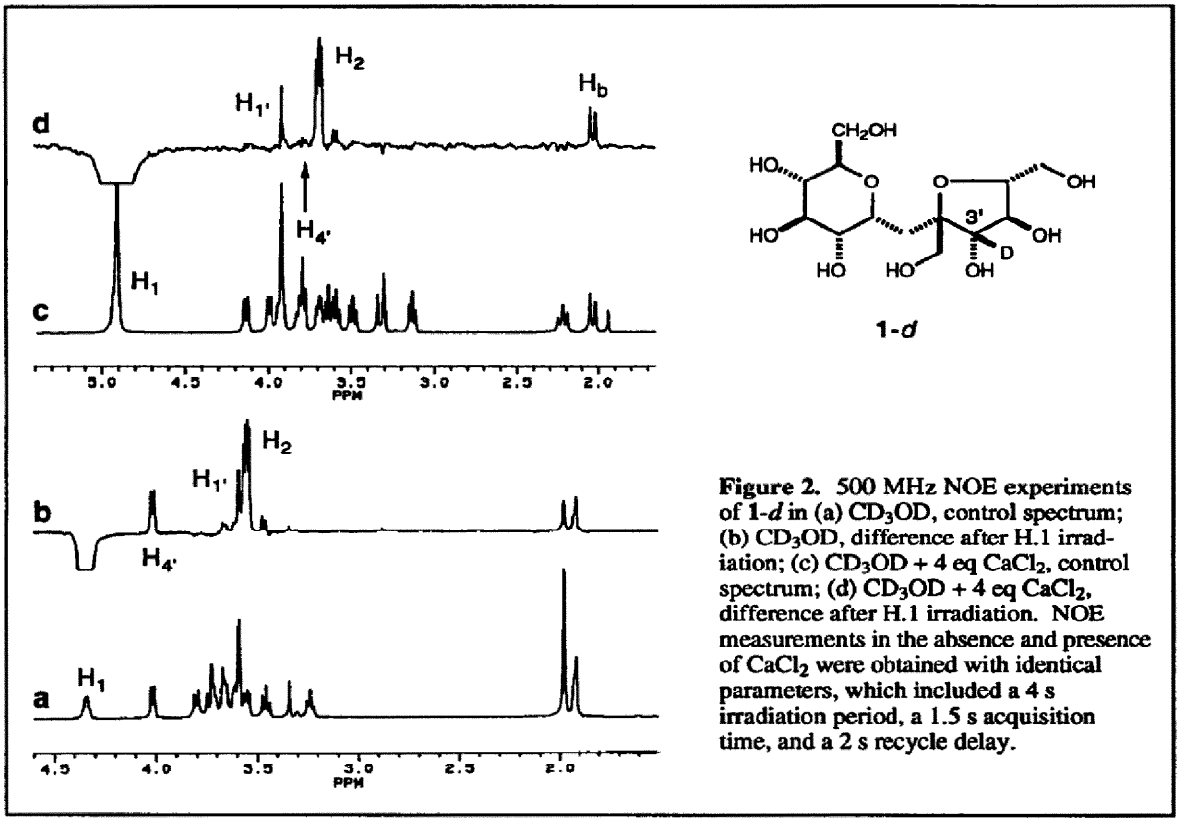


Figure 2. 500 MHz NOE experiments of **1-*d*** in (a) CD_3OD, control spectrum; (b) CD_3OD, difference after H.1 irradiation; (c) CD_3OD + 4 eq $CaCl_2$, control spectrum; (d) CD_3OD + 4 eq $CaCl_2$, difference after H.1 irradiation. NOE measurements in the absence and presence of $CaCl_2$ were obtained with identical parameters, which included a 4 s irradiation period, a 1.5 s acquisition time, and a 2 s recycle delay.

Several points concerning the NMR studies are noteworthy: (i) the Ca^{2+}-induced conformational changes in *C*-sucrose occur in methanol but not in aqueous solution, (ii) addition of several equivalents of $SrCl_2 \cdot 6H_2O$ induces Ca^{2+}-like chemical shifts on **1**; these chemical shift effects are still apparent with 10 eq Sr^{2+} and 60 eq H_2O, (iii) sodium (added as NaCl) in methanol produces small chemical shift effects in **1** but the glycosidic coupling constants did not change and the H.1-H.4' NOE was still evident, (iv) no effects were observed following the addition of Mg^{2+}, and (v) replacing the C.1' hydroxymethyl group in **1** with a proton appears to remove any affinity for Ca^{2+}, as no chemical shift effects were observed for this compound in methanol.

A qualitative measure of the different conformational behavior of *C*- and *O*-sucrose towards calcium in methanol is further evidenced by optical rotation data (Table 1). Within the limits of detection, the optical rotation of *O*-sucrose (**2**) does not change with the addition of up to 20 eq of Ca^{2+}. This behavior is consistent

with the reported inertness of **2** toward counterions, although an absence of change in rotation is not a suitable criterion for a lack of binding affinity.[3] In contrast, addition of 2 eq of Ca^{2+} to a *C*-sucrose solution causes the optical rotation to nearly double, with a limiting value reached after ca. 6 eq Ca^{2+}. This polarimetric behavior parallels the NMR chemical shift, coupling constant, and NOE evidence for a calcium-induced conformational reorganization in **1**.

Table 1. Optical Rotations[7] for *C*-Sucrose (**1**) and *O*-Sucrose (**2**)

Equivalents of Ca^{2+}	$[\alpha]_D$ **1**	**2**
0	+48.3°	+72.8°
2	+84.9°	+73.8°
6	+91.4°	+74.2°

In conclusion, these experiments have revealed that *C*-sucrose and *O*-sucrose respond differently, in a conformational sense, to the presence of certain divalent cations in dilute methanol solution. Given the following observations: (i) the pyranoside and furanoside rings in *C*- and *O*-sucrose have similar solution conformations, (ii) the ϕ/ψ degrees of freedom in both **1** and **2** appear similar, and (iii) the C.1-C.2' "anomeric center-anomeric center" distance in **1** and **2** are within 0.1 Å of each other[2], one rationale for the inertness of *O*-sucrose toward cations might be due to the lone pairs of electrons on the glycosidic oxygen atom itself. When this atom is replaced with a carbon atom, a substance emerges with a very different behavior towards certain cations. The comparison reported here has revealed that in the presence of certain ions, the conformational flexibility of a *C*-glycoside can be different from that of the parent substance, and this difference may become significant when considering *C*-glycosides as surrogates for natural products which interact with other molecules of biological relevance.

Acknowledgment. Financial support from the National Institutes of Health (NS-12108) and the National Science Foundation (CHE 89-09762) is gratefully acknowledged. D.J.O. thanks the National Science Foundation for a postdoctoral fellowship (CHE 91-02269). The NMR facility at Harvard University is supported by the NSF and the NIH.

References and Notes.

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2. O'Leary, D. J.; Kishi, Y. *J. Org. Chem.* **1993**, *58*, 304-306, O'Leary, D. J.; Kishi, Y. *Abstracts of Papers*; 33rd Experimental NMR Conference, Pacific Grove, CA, March, 1992.

3. Angyal, S. J. *Adv. Carbohydr. Chem. and Biochem.*, **1989**, *47*, 1-43.

4. 500 MHz ^{1}H NMR (**1**, CD_3OD): 1.92 ppm (H_a, H_b); 4.35 (H_1); 3.57 (H_2); 3.49 (H_3); 3.23 (H_4); 3.69 (H_5); 3.74 (H_{6a}); 3.61 (H_{6b}); 3.61 ($H_{1a'}$, $H_{1b'}$); 4.07 ($H_{3'}$); 4.01 ($H_{4'}$); 3.72 ($H_{5'}$); 3.80 ($H_{6a'}$); 3.67 ($H_{6b'}$). (**1**, CD_3OD/6 eq $CaCl_2$): 2.22 (H_a); 2.03 (H_b); 4.94 (H_1); 3.73 (H_2); 3.62 (H_3); 3.16 (H_4); 3.82 (H_5); 4.14 (H_{6a}); 3.49 (H_{6b}); 3.92 ($H_{1a'}$); 3.93 ($H_{1b'}$); 3.79 ($H_{3'}$); 3.79 ($H_{4'}$); 3.93 ($H_{5'}$); 3.99 ($H_{6a'}$); 3.63 ($H_{6b'}$).

5. Chemical shift difference (**1**, CD_3OD, δw/Ca^{2+} – δ w/o): + 0.30 ppm (H_a); + 0.11 (H_b); + 0.59 (H_1); + 0.16 (H_2); + 0.13 (H_3); – 0.07 (H_4); + 0.13 (H_5); + 0.40 (H_{6a}); – 0.12 (H_{6b}); + 0.31 ($H_{1a'}$); + 0.32 ($H_{1b'}$); – 0.27 ($H_{3'}$); – 0.22 ($H_{4'}$); + 0.21 ($H_{5'}$); + 0.19 ($H_{6a'}$); – 0.04 ($H_{6b'}$).

6. O'Leary, D. J.; Kishi, Y. Submitted for publication.

7. *C*-Sucrose : *c* 0.38, *O*-Sucrose: *c* 0.21.

(*Received in USA* 31 *May* 1994; *accepted* 10 *June* 1994)