Carbohydrate Polymers 3 (1983) 13–22

High Resolution Nuclear Magnetic Resonance Studies of Nigeran

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(Received: 24 March 1982)

SUMMARY

Polymer motion in solution can be studied by ^{13}C NMR relaxation methods, which provide information about the correlation time for C–H vectors. ^{13}C-Relaxation and Nuclear Overhauser Enhancement (NOE) data may frequently be combined to determine the dipole–dipole relaxation contribution. An alternative method is proposed based on a comparison of the proton spin-lattice relaxation rates of the centre proton resonances of an unlabelled molecule with the relaxation rates of the ^{13}C satellites (from ^{13}C labelled molecules).

Selectively labelled nigeran which is an alternating 1 → 3 and 1 → 4 α-D-glucan has been investigated. The discussion in terms of the occurrence of different motions for each of the two units of the polymer requires an unambiguous assignment of the two anomeric carbons. For this reason a detailed assignment of the ^1H and ^{13}C Nuclear Magnetic Resonance (NMR) spectra of nigeran in dimethylsulphoxide-d_6 is described, based on T_1 and NOE measurements in addition to selective homonuclear and heteronuclear spin decoupling experiments. These values are correlated with a conforma-

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Carbohydrate Polymers 0144-8617/83/0003-0013/$03.00 –

ation estimated by HSEA hard-spheres calculation. The measurements of the relaxation parameters for labelled and unlabelled compounds which provide an alternative determination of the ^{13}C-^{1}H dipole-dipole relaxation contribution in a macromolecule agree well with ^{13}C-{^{1}H} NOE experiments.

INTRODUCTION

The polysaccharide, nigeran, is a polyglucan in which the repeating unit consists of two α-D-glucopyranosyl residues: (—3-α-D-Glcp-1—4-α-D-Glcp-1—)$_n$. Perez *et al.* (1979) have studied anhydrous nigeran in the solid state and proposed the conformation shown in Fig. 1. ^{13}C NMR study of this polysaccharide has recently been published (Bobbit *et al.*, 1980) and differences in the intensities of the ^{13}C resonances were noticed as high as 20% based on the integration area.

To explain these differences, an extensive ^{1}H and ^{13}C NMR spectroscopic analysis has been carried out using both natural abundance and ^{13}C labelled nigeran for the ^{1}H and ^{13}C T_1 measurements and NOE experiments. Further, the possibility of obtaining biosynthesised polysaccharides from ^{13}C labelled monomers enabled ^{13}C–^{1}H dipolar interactions to be accurately determined and thereby extended the method already published for labelled monosaccharides by London *et al.* (1977) to polysaccharides.

EXPERIMENTAL SECTION

^{13}C enriched nigeran labelled at C-1 (56%) was prepared from D-glucose (^{13}C-1) as described by Bobbit *et al.* (1980).

The ^{1}H NMR spectra were obtained at 270 MHz on a Bruker HX-270 instrument using 2% solutions in DMSO-d_6 at 360 K with the solvent as an internal reference (2·50 ppm). The use of a spectral width of 3 kHz, with a data memory of 32 K gave a digital resolution of ± 0·2 Hz. The pulse width used was 15 μs (90°). Relaxation data were obtained by the inversion recovery method with 10 different τ values and employed the initial slope approximation. The accuracy is considered to be ± 5%.

The ^{13}C NMR spectra were obtained at 67·89 MHz on the same instrument using 8% solutions in DMSO-d_6 at 360 K with the solvent as an internal reference (δ = 39·45 ppm). The use of a spectral width of

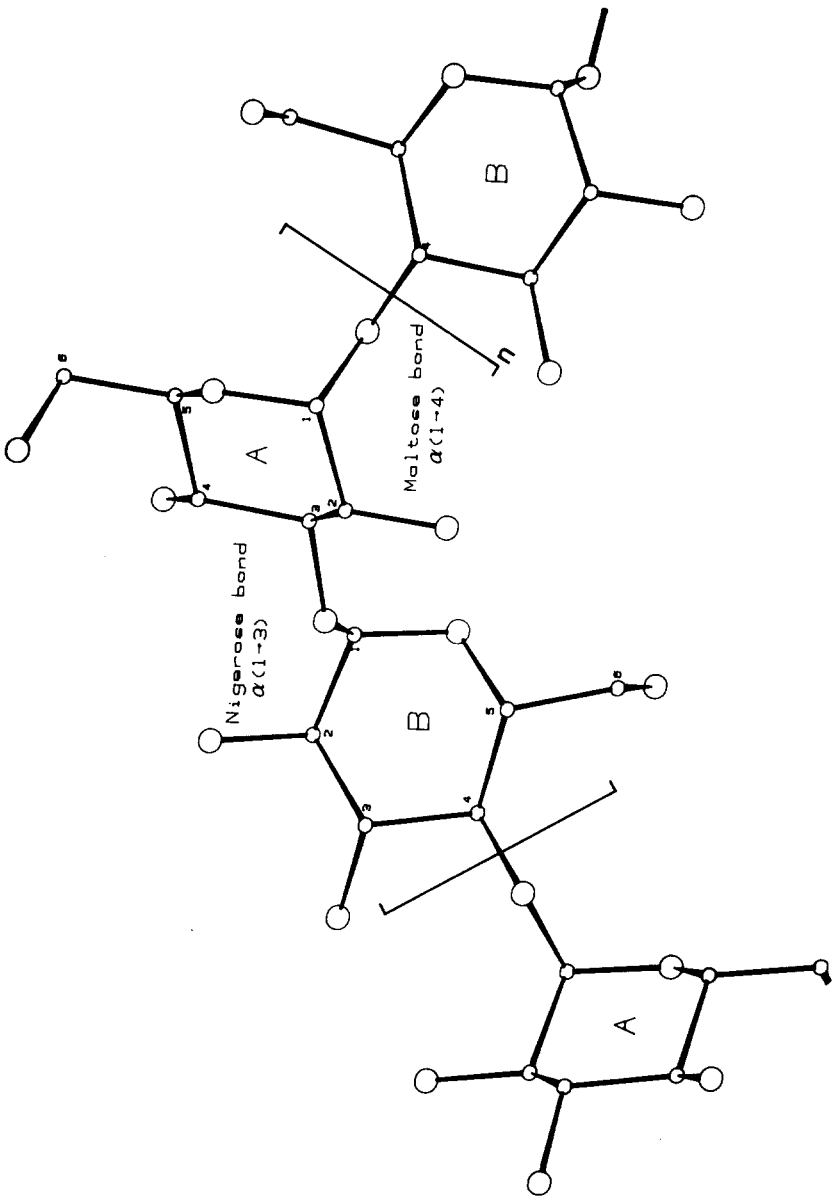

Fig. 1. Solid state conformation of anhydrous nigeran.

10 kHz with a data memory of 32 K gave a digital resolution of ± 0·6 Hz. The pulse width used was 12 μs (90°). Relaxation data were obtained by the inversion-recovery method $(180°-\tau-90°-T)_n$ with six different τ values using the initial slope approximation where the delay time between experiments (T) was adjusted so that $T > 5_{max} T_1^{obs}$.

The data were fitted to an exponential curve by a 3-parameter fit using the expression given by Saas & Ziessow (1977). The accuracy of the relaxation data is considered to be ± 5%.

The $^{13}C-\{^1H\}$ NOE values were determined using a gated technique described by Canet (1976).

RESULTS AND DISCUSSION

The ^{13}C spectrum has previously been partially assigned by Bobbit *et al.* (1980) but the interpretation of the results required a non-ambiguous assignment of the two anomeric carbons. Therefore a complete analysis of the proton spectrum has been carried out.

The NMR study has been performed in DMSO-d_6 due to poor solubility of nigeran in water. One drop of D_2O has been added in order to avoid additional complications from the spin–spin couplings of the hydroxyl protons.

An unambiguous assignment of the 14 proton signals, 12 of which resonate between 3·3 and 4·0 ppm has been performed using $^1H-\{^1H\}$ homonuclear and $^{13}C-\{^1H\}$ heteronuclear spin decoupling techniques, partially relaxed spectra and $^1H-\{^1H\}$ NOE experiments.

The ^{13}C chemical shifts of C-3A and C-4B (see Fig. 1) could be assigned with certainty based on comparison with model compounds. Selective $^{13}C-\{^1H\}$ decoupling then gave the chemical shifts for the corresponding protons H-3A and H-4B.

$^1H-\{^1H\}$ NOE experiments between H-1A to H-4B and H-1B to H-3A across the glycosidic linkage allow a determination of the H-1A and H-1B signals. Homodecoupling experiments now allow a determination of H-2A, H-2B, H-3B and H-4A. Partially relaxed spectra with, in addition, homonuclear decoupling experiments, give the assignments for the faster relaxing H-6 protons and the H-5 protons. It is only possible to assign the two systems H-5B, H-6B, H-6′B and H-5A, H-6A, H-6′A based on a selective $^{13}C-\{^1H\}$ decoupling assuming that C-5B resonates at a higher field than C-5A which is in agreement with data from model compounds. The results are presented in Table 1 together

TABLE 1

^{1}H Chemical Shifts[a] and Coupling Constants[b]

	H-1	*H-2*	*H-3*	*H-4*	*H-5*	*H-6$_S^c$*	*H-6$_R^c$*
A-Unit	5·13	3·42	3·60	3·43	3·6–3·7	3·63	3·52
	(J_{12} = 3·5)	(J_{23} = 9·0)	(J_{34} = 9·0)		(J_{56S} = 5·5)	(J_{56R} = 5·5)	(J_{6S6R} = 10·5)
B-Unit	5·04	3·35	3·78	3·4–3·5	3·92	3·71	3·62
	(J_{12} = 3·5)	(J_{23} = 9·0)					
α-Me-Glc	4·54	3·23	3·43	3·11	3·37	3·65	3·49
	(J_{12} = 3·6)	(J_{23} = 9·6)	(J_{34} = 9·6)	(J_{25} = 9·6)	(J_{56S} = 2·3)	(J_{56R} = 5·6)	(J_{6S6R} = 11·6)
α-Cyclodextrin	4·87	3·37	3·65	3·37	3·63	3·45 to	3·85

[a] Measured at 270 MHz at 360 K as 2% solutions, δ values in ppm relative to DMSO-d_6 as internal reference (δ = 2·50 ppm).

[b] Coupling constants (in Hz) given in brackets.

[c] Relative assignment of H-6_S (pro-S) and H-6_R (pro-R) based on the assumption that J_{56S} is small (2·5 Hz) and J_{56R} larger (5·5 Hz).

with the data from the methyl α-D-glucopyranoside and α-cyclodextrin in DMSO-d_6. The data show that H-5B is shifted downfield $\simeq 0.3$ ppm relative to the values in the A-unit. This agrees with a conformation calculated using HSEA hard spheres calculations as in Lemieux *et al.* (1980, 1982) which give ψ_M, ϕ_M* values for 1-4 linkage of $-16°$ and $-22°$ ($\pm 5°$) and ψ_N, ϕ_N* values for the 1-3 linkage of $-37°$ and $-18°$ ($\pm 5°$). In this conformation, H-5B is placed at 2·54 Å from the oxygen (O-2A) of the A-unit which accounts for the observed deshielding of H-5B. The calculated conformation is also in good agreement with the results obtained in the solid state by Perez *et al.* (1979) (ψ_M, ϕ_M: $-15.3°$, $-13.4°$, ψ_N, ϕ_N: $-30°$, $-18.5°$ respectively).

The ^1H T_1 relaxation values for the anomeric protons H-1A and H-1B are shown in Fig. 2 for both unlabelled and ^{13}C labelled compound. It is seen that the H-1B proton is relaxing somewhat slower than the H-1A in both cases (see Table 2 for values). This agrees with the calculated conformation which places H-1A closer to H-4B than H-1B to H-3A (2·18 and 2·35 Å respectively) and this offers a more efficient relaxation for H-1A than H-1B.

TABLE 2
Proton Relaxation Values (S) of Anomeric Protons[a] of ^{13}C-labelled Nigeran in DMSO-d_6 at 360 K

	H-1A ^{13}C	*H-1B ^{13}C*	*H-1A ^{12}C*	*H-1B ^{12}C*	*H-1A ^{13}C*	*H-1B ^{13}C*
^{13}C Labelled sample[b]	0·26	0·28	0·68[c]	0·71[c]	0·26	0·30

[a] The entries H-1A ^{13}C and H-1B ^{13}C refer to the two sidebands of the H-1 signals in the ^{13}C-labelled molecule. The accuracy of the relaxation data is considered to be ± 5%.

[b] Statistically labelled 55% in C-1A and C-1B, 10% in C-6A and C-6B.

[c] 0·69 for H-1A and 0·75 for H-1B in unlabelled compound.

* The glycosidic torsion angles being defined as follows: $\phi_M = H\text{-}1_A\text{–}C\text{-}1_A\text{–}O_{AB}\text{–}C\text{-}4_B$; $\psi_M = C\text{-}1_A\text{–}O_{AB}\text{–}C\text{-}4\text{–}H\text{-}4_B$ for maltose bond; $\phi_N = H\text{-}1_B\text{–}C\text{-}1_B\text{–}O_{BA}\text{–}C\text{-}3_A$; $\psi_N = C\text{-}1_B\text{–}O_{BA}\text{–}C\text{-}3_A\text{–}H\text{-}3_A$ for nigerose bond.

From the resuts with the ^{13}C labelled compound it is seen that the ^{13}C nuclei offer an additional relaxation mechanism for the anomeric protons as shown in Fig. 2. It is therefore possible to determine the ^{13}C–^{1}H dipolar relaxation contributions as already shown by London *et al.* (1977) for simple monosaccharides. A calculation using these values together with the ^{13}C T_1 values shown in Table 3 establishes that the relaxation of the C-1A nuclei is 45% dipolar in nature and 55% by other mechanisms. Similar calculations for the C-1B nuclei give values of 38% by dipolar and 62% by other mechanisms. It is furthermore possible to calculate the ^{13}C–^{1}H NOE from these values and compare with the experimentally obtained values. For the A-unit an NOE of 1·85 is calculated which compares well with the measured value of 1·78. For the B-unit the values are 1·87 and 2·02 respectively.

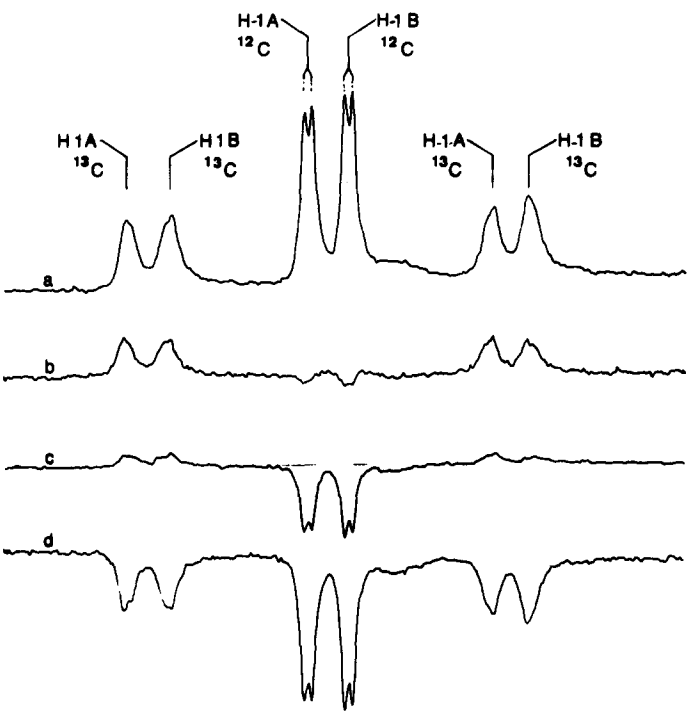

Fig. 2. Partially relaxed anomeric proton spectra of labelled nigeran (55% of ^{13}C in C-1); a, $\tau = 3{\cdot}5$ s; b, $\tau = 0{\cdot}4$ s; c, $\tau = 0{\cdot}2$ s; d, $\tau = 0{\cdot}001$ s.

TABLE 3
Chemical Shifts,[a] T_1 Values[b] and NOE Values for Nigeran

	C-1A	*C-2A*	*C-3A*	*C-4A*	*C-5A*	*C-6A*	*C-1B*	*C-2B*	*C-3B*	*C-4B*	*C-5B*	*C-6B*
δ values[a]	101·0	71·4	83·2	70·3	73·5	61·3	100·0	72·7	73·9	79·8	71·4	60·9
T_1 values[b]	0·193	0·189	0·185	0·181	0·190	0·145	0·186	0·216	0·222	0·204	0·189	0·120
NOE	1·80	1·90	1·70	1·95	1·70	2·00	1·75	2·10	1·95	2·00	1·90	1·60
	2·00[c]					2·45[c]	1·85[c]					2·30[c]
T_1 values[d]	0·34	0·33	0·34	0·31	0·34	0·20	0·42	0·39	0·39	0·38	0·40	0·21

B-unit indicate reducing end of the molecule.

[a] Chemical shifts relative to internal dioxane (δ = 67·40 ppm) obtained at 67·89 MHz at 360 K.

[b] Values in seconds, average of three experiments. The accuracy is considered to be ± 5%.

[c] NOE values for the ^{13}C-labelled compound (C-1 or C-6).

[d] T_1 values for 0·5 M solution of methyl β-D-maltopyranoside in D_2O at 306 K determined at 67·89 MHz.

^{13}C–{^{1}H} selective heteronuclear spin decoupling experiments based on the above mentioned ^{1}H-NMR data confirm the ^{13}C NMR assignments given by Bobbit *et al.* (1980) and allow an assignment of the C-6 signals which has not been possible previously (Table 3). The latter assignment was further confirmed by a selective population transfer experiment as in Pachler & Wessels (1973) with inversion of the ^{13}C-satellites of H-6B of the labelled compound in the proton spectrum which gives an 'INDOR' response of the high field carbon (C-6B) in the ^{13}C NMR spectrum.

From Table 3 is seen a small but significant difference in the ^{13}C T_1 values of the two units, the larger values arising from the B-unit with the exception of the C-1 and C-6 carbon atoms. This is in agreement with a preferential axis of motion through the B-unit which places the C–H vectors of the A-unit at a different angle to the principal axis of motion and thus gives different T_1 values, consistent with the published data of Czarniecki & Thornton (1977) and Berry *et al.* (1977). Similar relaxation data are observed for the ^{13}C T_1 values of methyl-β-D-maltopyranoside (Table 3) in water. This model also offers an explanation for the differences in the T_1 values of the anomeric carbon atoms. In the above calculated conformation the $C\text{-}1_B$–$H\text{-}1_B$ vector is almost parallel to e.g. the $C\text{-}3_A$–$H\text{-}3_A$ vector and similarly the $C\text{-}1_A$–$H\text{-}1_A$ vector is almost parallel to e.g. the $C\text{-}4_B$–$H\text{-}4_B$ vector. It is therefore reasonable to expect ^{13}C T_1 values for the C-1B similar to the values found for the A-unit as seen from Table 3.

The large difference in the T_1 values for the C-6 carbons is most likely caused by a different internal motion of the hydroxymethyl group relative to the overall motion of the molecule, with the C-6A carbon being less hindered in its internal motion relative to the C-6B carbon atom and the overall segmental motion of the polysaccharide.

CONCLUSION

Complete assignment of the ^{1}H and ^{13}C spectra of nigeran in DMSO-d_6 has been performed using different experimental techniques such as homonuclear, heteronuclear spin decoupling, and T_1 and ^{1}H–{^{1}H} NOE measurements. These results are in good accordance with the conformation predicted from HSEA (hard sphere) calculations. T_1 relaxation values of the anomeric protons H-1A and H-1B indicate that the H-1B

proton is relaxing somewhat faster than H-1A which is in agreement with the calculated conformation. The relaxation rate of these protons is considerably increased for a ^{13}C enriched nigeran sample through the additional ^{13}C–^{1}H dipolar relaxation contribution which is found to be 45% of the total. Calculated results of the ^{13}C–^{1}H Nuclear Overhauser Enhancement (NOE) from these values are in good agreement with the experimental values.

It has been possible to rationalise the ^{13}C relaxation data based on a model which predicts a principal axis of motion for the molecule through the B-ring in analogy with data obtained for methyl-β-D-maltopyranoside. The internal motions of the two hydroxymethyl groups (C-6A and C-6B) are very different; the C-6A carbon has a faster rotational motion than C-6B, this, possibly, being correlated with the position of this group being external relative to the main chain.

ACKNOWLEDGEMENTS

The NMR spectrometer used in this investigation was provided by the Danish Natural Science Research Council who also kindly supported one of us (M. Vignon) during a visit to Denmark in July and August 1979.

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