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NUCLEAR MAGNETIC RESONANCE OF LICHENIN

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Lichenin, a polysaccharide occuring in Iceland moss (Cetraria islandica) was first isolated by KLASON (1) ; methylation studies showed it to contain glucose units linked by β(1→4) and β(1→3) glycosidic linkages in a ratio of 2:1 (2) (3). This was confirmed by chemical and enzymic hydrolysis (4) (5) (6).

This paper deals with the relative arrangement of the two types of linkages, and the conformation of the polymer in solution using proton n.m.r. as an investigation method. Since lichenin is a linear copolymer containing nearly 65 to 70 % of β(1→4) linked glucose units and 30 to 35 % of β(1→3) linked ones (4) (5) (6), n.m.r. can show if the copolymer is a random or regular one (the possibility of a block copolymer is eliminated by the absence of β(1→3) oligomers on hydrolysis).

N.m.r. spectra of polysaccharides provide information first of all on the configuration of the glycosidic linkage, through the chemical shift and the coupling constant of the anomeric proton H(1). The anomeric proton, for a glucopyranose, shows a doublet at higher field for an α linkage at lower field with a higher coupling constant value (J_{H1-H2} = 7 to 8 Hz) for a β linkage. Since n.m.r. studies of klebsiella polysaccharides showed different H(1) protons for each different sugar unit in the sequence of the regular copolysaccharide (7), it seems that n.m.r. spectra can be a good indication of the regularity of the polymer through the anomeric signals. In the case of a random distribution of β(1 → 4) and β(1 → 3) linkages in lichenin broad signals are to be expected. Table I gives the possible arrangement of D-glucopyranosyl units and the relative intensity of anomeric protons H(1) in case of random or regular copolymer taking into account that 33 % of glycosidic linkages in lichenin are β(1 → 3).

TABLE I

Relative arrangement of D-glucopyranosyl units in lichenin		Relative intensity of H(1)	
		Random	Regular
(1)	β(1→4) linkage substitued on 4 position	4	3
(2)	β(1→4) linkage substitued on 3 position	2	3
(3)	β(1→3) linkage substitued on 4 position	2	3
(4)	β(1→3) linkage substitued on 3 position	1	0

Lichenin was extracted by the procedure of PEAT (5) and PERLIN (6) from Iceland moss (Pennick CO. N.Y.) purified through the triacetate and regenerated by a 0.2 M sodium methylate solution. The regenerated lichenin was then dissolved in hot water and freeze-dried.

250 MHz n.m.r. spectra of lichenin in dimethyl sulfoxyde-d_6 solution at 20°C show broad signals between δ = 5.6 and 2.8 ppm. Spectra were run with an internal HMDS capillary reference, chemical shifts have been corrected. Spectrum of lichenin which had been previously exchanged three times in hot D_2O and then freeze-dried, showed only one remaining signal downfield δ = 4 ppm. This signal was attributed to the anomeric protons with a β configuration. The broad shape of the signal with a half width of 20 Hz could be attributed to the possibility of two adjacent anomeric protons, H(1) $\beta(1 \rightarrow 4)$ and H(1) $\beta(1 \rightarrow 3)$ both having a coupling constant of about 8 Hz. In fact spectrum given by Figure 1 of the exchanged sample at 100°C shows clearly the presence of three doublets at 4.34, 4.29 and 4.25 ppm, with a coupling constant of 7.5 Hz each, charasteristic of a β configuration. The intensity of each doublet being approximately equal (the ratio of the integrals of the more shielded doublet at 4.34 ppm to the two other ones at 4.29 and 4.25 being exactly 3/7). It is now usual to observe different anomeric proton signals for sugar units having different substituent positions (7) (8), however to our knowledge this is the first time that two glucopyranosyl units both 4 substitued give two different n.m.r. signals. It is worth noting that 100 MHz spectrum, even at 100°C, or the 250 MHz at room temperature can lead to a wrong interpretation, due to the fact that the anomeric signal looks like two adjacent doublets. Due to the small chemical shift difference of the three doublets, no definitive conclusion can be drawn of their value, but it must be noticed that no doublet occurs at δ = 4.44 ppm chemical shift found for the anomeric proton of laminarin in the same conditions (9) (case 4 of table I).

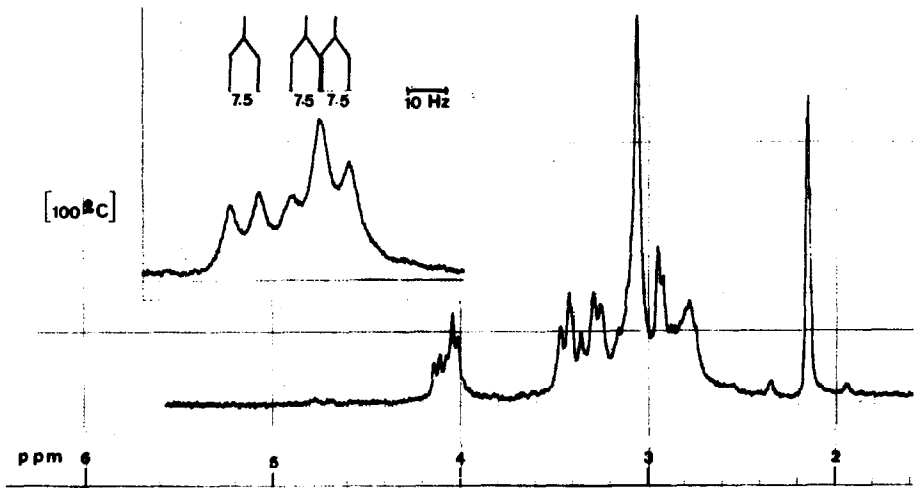

Figure 1 : The 250 MHz spectra of lichenin (HMDS external reference)
Inset : anomeric protons enlargement.

The observation of three kinds of linked glucose units in the n.m.r. spectra leads us to postulate a regular copolymer in which the repeating unit is given by figure 2. This sequence is in agreement with the results of PERLIN (6) based on enzymic degradation by cellulase in which the major product was the trisaccharide O-β-D-glucopyranosyl (1 → 3)-O-β-D-glucopyranosyl (1 → 4)-D-glucopyranose. On the basis of this sequence the n.m.r. spectra should show twenty one different ring protons.

The spectrum of the non-exchanged lichenin sample showed downfield at δ = 4 ppm, three broad signals at 5.36, 4.74, 4.40 ppm of relative intensity 1/2/1. The signals at 5.36 and 4.74 disappeared after D_2O exchange and must be attributed to OH signals. CASU et al. (9) first suggested that OH protons with peaks downfield of δ = 5 ppm in the cellobiose spectrum could be attributed to OH(3) which was intramolecularly hydrogen bonded to the ring oxygen of the adjacent glucopyranosyl unit. This behaviour has been confirmed for cellobiose (10) and cellotetraose (11). Infrared studies on cellulose have consistently supported this interpretation (12).

Spectra recorded at different temperature between 20°C and 100°C showed a faster upfield shift for the signal at δ = 5.36 ppm than for the one at δ = 4.74 ppm indicating a difference between two types of hydroxyl; this difference could be explained (13) by an internal hydrogen bond for one kind of hydroxyl.

Based on the trisaccharide sequence of figure 2, it means that three OH signals (δ = 5.36 ppm) would be expected to be hydrogen bonded : OH(3) being probably bonded with ring oxygens of adjacent units and OH(4) most probably being hydrogen bonded in the same way, based on conformational energy calculations of laminarin (14). The six remaining signals (δ = 4.74 ppm) being attributed to non-hydrogen bonded hydroxyls OH(2) and OH(6) of the trisaccharide sequence. The n.m.r. spectrum of the triacetate in $CDCl_3$ solution showed a complex pattern without structure for the ring protons, even at high temperature ; the most shielded signal at 5.1 ppm was attributed to H(3-OAc) by comparison with the cellulose triacetate spectrum (15). The acetate part gave two broad signals at 1.99 and 2.13 ppm and a narrow one at 2.07 ppm. The broad signals are caused by the superposition of different kinds of acetyl groups. This fact would be in keeping with the trisaccharide sequence in which 21 ring protons and 9 acetyl groups are possible.

Figure 2 shows that lichenin could have a ribbon-like conformation with at least 3 glucose units in each period, H(1A) and H(1C) being on one side of the ribbon, and H(1B) on the other. The chemical shift difference between H(1C) and H(1A) or H(1B) is easily explained by the influence of the position of the substitution. The chemical shift difference between H(1A) and H(1B) is no doubt due to their different relative environment. Nevertheless a difference in chemical shift due simply to the fact that H(1B) is attached to O-β-D-glucopyranosyl (1 → 4) and H(1A) to O-β-D glucopyranosyl (1 → 3) units cannot be entirely ruled out.

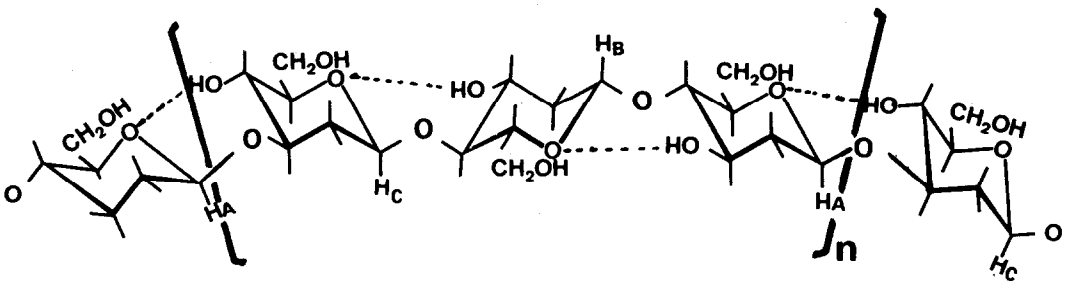

Figure 2 : Proposed structure of lichenin

Lichenin was extracted in the laboratory of Prof. A.S. PERLIN by one of us (M.V.). We are indebted to Mr REUTENAUER for obtaining the 250 MHz spectra (Cameca 250).

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