# CARBON-13 NMR STUDY OF SACCHARIDES*

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The C-13 nmr studies of mono-, oligo-, and polysaccharides are reviewed. Especially, the procedures of structural determination of polysaccharides by carbon magnetic resonance are described.

In the last decade, the nmr instrumentation has been strikingly developed. One is a high field 84.6 kG (360 MHz for proton) instrument with a super-conducting magnet, and the other is a pulsed Fourier transform (FT) instrument combined with a minicomputer. This FT nmr instrument has made possible to take nmr spectra of low natural abandant magnetic nuclei such as $^{13}C$, $^{15}N$, and so on.[1] In the case of biomacromolecules, high field proton magnetic resonance such as 360 MHz cannot afford well-resolved spectra because of its small-range chemical shift ( about 10 ppm). On the contrary, carbon magnetic resonance gives finely resolved spectra even at low magnetic field such as 14 kG (15 MHz), because its chemical shift range is about 200 ppm.[2,3] This wide chemical shift range makes cmr be very susceptible to conformational and configurational

changes of molecule. Especially, the cmr application to polysaccharide research is most suitable, because its structural determination is rather complicated and needs a lot of labor in spite of its relatively simple and repeating structure.[4]

The cmr studies of carbohydrates were firstly reported in 1969.[5 - 7] In 1970, the cmr spectra of pento- and hexo-aldopyranoses were assigned as seen in Stothers' book.[8,9] The cmr study of oligosaccharides was firstly reported by Neuss et al on antibiotic pseudo-disaccharides, hygromycins.[10] The author's group showed that α- and β-linkage anomeric signals of glucopyranobioses appear at distinctly different field-strength in neutral conditions (97 - 101 ppm downfield from $Me_4Si$ for α, and 103 - 105 ppm for β).[11] Also, Dorman and Roberts,[12] Doddrell and Allerhand,[13] and Voelter et al[14, 15] showed the cmr of some common oligosaccharides and polysaccharides. These studies clearly showed the utility of cmr spectra for the structural determination of carbohydrates.

The authors studied the cmr spectra of all mono-O-methyl glucopyranoses, all glucopyrnobioses, and selected glucopyranotrioses.[16] The methylation or β-glucosidation shift on a linkage carbon is 8 - 10 ppm downfield from that of unsubstituted glucose and the α-glucosidation shift is 3 - 7 ppm downfield due to the α-axial steric compression. The chemical shifts of linkage carbons in Table 1 are almost same throughout oligosaccharides to glucans in neutral conditions.[17 - 21] The shift of β-carbon due to methylation or glucosidation is

0.5 - 2 ppm upfield except the effect on C-1 by O-2 methylation or glucosidation. The effect on other carbons is negligible. Similar effects were shown in the case of mannopyranoside series by Gorin et al with all mono-O-methyl mannopyranoses and some specifically deuterated mannopyranobioses and mannans.[22] Colson and King, also, studied the cmr of disaccharides containing rhamnose as a model of immunological polysaccharides.[23] Other research groups got similar results on oligosaccharides.[20, 21, 24 - 26]

The continuous wave (CW) cmr spectra of glucans were measured firstly by Dorman and Roberts[12] and then by the author's group.[16] However, a CW instrument, a usual machine with a time-averaging signal accumulator, gave only poor spectra and is very limited for the research of polysaccharide. On the contrary, the FT method has given finely resolved spectra of polysaccharides. In 1972, the first two reports by the FT method appeared on mannans by Gorin and Spencer[27] and on heparin by Perlin et al.[28] In 1973, Jennings and Smith reported the cmr of glucan containing α-1,6- and α-1,4-linkages,[18] and the author's group showed the cmr and pmr study of glucan containing α-1,6-, α-1,2-, and α-1,3-linkages.[17] The spectral assignment of this glucan obtained from Leuconostoc mensenteroides NRRL B-1299 will be shown as an example. This glucan showed anomeric signals at 99.4, 98.0, and 97.2 ppm, all of which are assigned to α-anomeric carbons. By comparison with Table 1, signals of linkage carbons

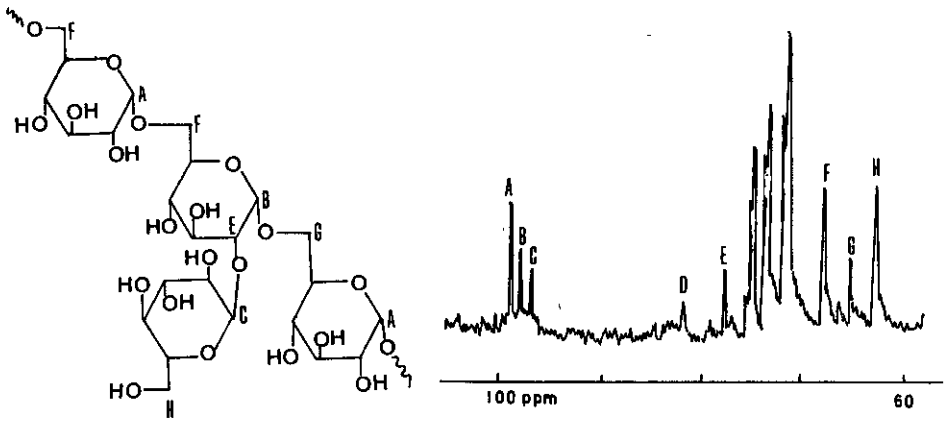



Fig. 1

appeared at 83.7, 77.7, 67.6, and 64.9 ppm assigned to C-3, C-2, C-6, and C-6 binding to an anomeric position of glucose having linked O-2, repectively. The anomeric signal at 99.4 ppm can be assigned to α-1,6 and α-1,3-linkages, that at 98.0 ppm to α-1,6-linkage adjacent to linked O-2, and that at 97.2 ppm to α-1,2-linkage. (Fig. 1) The intensity ratio of those anomeric signals was as same as the result of chemical degradation. Thus, in the case of glucan, cmr spectra are easily assigned with Table 1. The authors, also, assigned the cmr spectra of glucans containing various linkages obtained from endosperm of naked barely, from Lentinus edodes (mashroom, Japanese name "shiitake"), from rabbit liver, from oyster,[29] and from Streptococcus mutans JC-2 (dental caries bacterium).[30]

Colson et al suggested that the downfield shift of signals of intersugar-linkage carbons of cycloamyloses and the pH-dependent downfield shift of those of amylose (α-1,4) and α-1,3

Table 1. Carbon chemical shift of anomeric and linkage carbon of glucan (ppm downfield from $Me_4Si$).

| linkage | anomeric configuration | | Chemical shift | |
|---|---|---|---|---|
| | a | b | anomeric | linkage |
| 1,2 | α | α | 97.5 | 77.1 |
| | | β | 99.0 | 79.1 |
| | β | α | 103.9 | 82.1 |
| | | β | 105.0 | 82.8 |
| 1,3 | α | α | 99.8 | 80.8 |
| | | β | | 83.2 |
| | β | α | 103.9 | 84.2 |
| | | β | | 86.7 |
| 1,4 | α | | 101.0 | 78.5 |
| | β | | 103.9 | 80.1 |
| 1,6 | α | | 99.4 | 67.4 |
| | β | | 103.8 | 70.2 |

a anomeric configuration of glucosyl residue.

b anomeric configuration of glucose residue having a linkage carbon.

c α-Glucoside shows its carbon signals between 71 and 74.4 ppm except C-6 (62 ppm) and C-1 (100 ppm) and β-glucoside does between 71 and 77.5 ppm except C-6 (62 ppm) and C-1 (105 ppm).

glucan (laminarin) are due to their conformational changes.[19] The disappearance of the C-13 signals of carrageenan in gel state was rationalized by Bryce et al in terms of formation of double helix.[31] Saito et al interpreted carbon spectral changes of some glucans depending on pH, concentration, and solvent with the above two discussions.[32 - 34] Seymour et al reported the cmr of microbial glucans and showed that there is the temperature dependent 0.017 ppm per degree upfield shift on each carbon of glucans.[35] The 62.8 MHz cmr spectrum of linchenine, β-1,3- and β-1,4-glucan, was studied by Gagnarie and Vincendon.[36] A surprising study was reported by Kainosho and Ajisaka. That is, boiled tissues of potato, corn kernels, and chestnut clearly showed carbon signals of starch, nevertheless these intact tissues did not.[37]

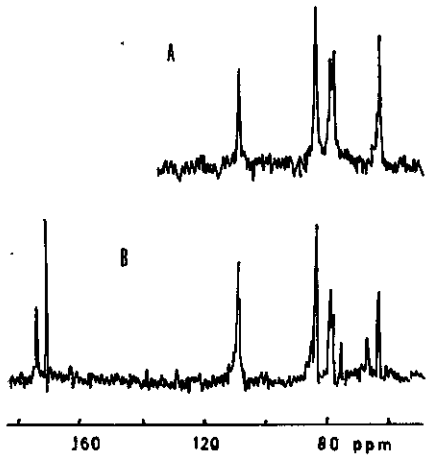
Since aldofuranosides are constituents of polysaccharides, the cmr study of them, also, is very important. Originally, carbon signals of ribofuranoses were detected by Hall and Johnson,[4] but they did not assign them. Afterwards, Breitmaier et al assigned the all signals of ribo-pyranoses and -furanoses.[38, 39] Jones et al reported the cmr assignment of phenyl β-D-ribofuranoside in their ribonucleoside study.[40] Their assignment was corrected by Mantsch and Smith[41] and by the authors.[42] The author s group reported the cmr assignment of methyl α- and β-D-ribofuranosides and methyl α- and β-L-arabinofuranosides for the purpose of structural determination of furanans along with the anomeric assignment of a furanan

obtained from coffee bean.[43] Ritchie et al studied the cmr of various cyclopentanols and of methyl aldofuranosides.[44] They corrected our assignment of methyl β-arabinoside. Gorin and Mazurek confirmed those results with specifically deuterated furanosides.[45] They also assigned the cmr of various methyl mono-O-methyl and mono-O-isopropyl furanosides as a model of polysaccharide.[46] The methylation shift of linked carbons of furanosides shows 8 - 10 ppm downfield shift, but the isopropylation shift of those carbons shows 5 - 6 ppm downfield shift. These on β-carbons are 0.5 - 2 ppm upfield shift and these of other carbons are less than 1 ppm, as these of pyranosides. Anomeric signals of furanosides appear at lower field than those of pyrnosides, and that of cis-1,2 configuration appears at 103.5 ppm and that of trans-1,2 does at 109 ppm.[44]

The structural determination of a malonogalactan obtained from Penicillium citrinum Thom 1131 by cmr will be shown as a successful example.[47] The carbon chemical shifts of the malonogalactan and its de-esterified galactan are listed in Table 2. By comparison with the chemical shifts of methyl α- and β-D-galactofuranosides,[45] the anomeric signals of both of the galactans at 108 ppm clearly suggest that they have β-anomeric linkage. The other signals of the de-esterified galactan at 83, 77.5 and 62.5 ppm and the signal at 78 ppm instead of 72 ppm suggest that the galactan has furanoside rings substituted at O-5, since the glycosidation effect makes

Table 2. Carbon chemical shift of malonogalactan and its de-esterified galactan from Penicillium citrinum Thom 1131.[47]

| | malono-galactan | galactan |
|---|---|---|
| C-1 | 108 | 108 |
| C-2 | 83 | 83 |
| C-3 | 83 and 77.5 | 77.5 |
| C-4 | 83 | 83 |
| C-5 | 78 | 78 |
| C-6 | 62.5 | 62.5 |
| ester C=O | 171.5 | |
| acid C=O | 174 | |
| methylene | 66 and 76 | |




(A) the galactan
(B) the malonogalactan

Table 3. Carbon chemical shift of furanosides.

| | C-1 | C-2 | C-3 | C-4 | C-5 | C-6 |
|---|---|---|---|---|---|---|
| methyl α-D-galactofuranoside[a] | 103.1 | 77.4 | 75.5 | 82.3 | 73.7 | 63.4 |
| methyl β-D-galactofuranoside[a] | 109.2 | 81.9 | 77.8 | 84.0 | 72.0 | 63.9 |
| methyl α-L-arabinofuranoside[b] | 109.2 | 81.8 | 77.5 | 84.9 | 62.4 | |
| methyl β-L-arabinofuranoside[b] | 103.2 | 77.4 | 75.4 | 82.9 | 62.4 | |

a data from ref. 45. b data from ref. 44.

6 ppm downfield shift on C-5, and about 2 ppm upfield shift on C-4 and -6 as above mentioned. The malonogalactan shows the more intense signal at 83 ppm and the less intense signal at 77.5 ppm than those of the galactan. This fact suggests that malonic acid attaches on O-3, and that the signal of C-3 binding to malonate ester shifts from 77.5 to 83 ppm.[48] The signal at 171.5 ppm due to ester carbonyl is more intense than the signal at 174 ppm due to free carboxyl. Moreover, the methylene signals at 66 and 76 ppm, which are extremely low field for ordinary α-carbon of malonate ester,[48] are the average chemical shift of keto-enol tautomer and are assigned to monoester and diester, respectively. Since this malonogalactan is very soluble in water (more than 500 mg/ml), the diester linkages should be intrachain but not interchain. Gorin and Mazurek studied the cmr of galactofuranotetrose from Penicillium charlesii galactomannan and obtained a similar result.[46] Joseleau et al reported the cmr study of water soluble arabinofuranans obtained from Rosa glauca.[49]

The cmr of mannans was studied extensively by Gorin et al.[27, 50 - 52] However, it is hard to determine those anomeric configurations with chemical shift, because α- and β-anomeric signals of mannans appear at the almost same field. Since the carbon-proton coupling constant ($^1J_{C-H}$) between anomeric carbon and proton is 170 Hz for an axial proton and 160 Hz for an equatorial proton.[6, 53 - 59] The $^1J_{C-H}$ value is a good criterion of the anomeric configuration of pyranosides and of mannans.

Many other cmr studies on polysaccharides have been reported (condroitin,[60 -62] heparin,[28, 63] menningococcal polysaccharides,[64 - 66] rhamnomannans,[67, 68] and others[69 - 73]). Also, several reviews have been published.[74 - 76]

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