

Björndal H, Hellerqvist C G, Lindberg B & Svensson S. Gas-liquid chromatography and mass spectrometry in methylation analysis of polysaccharides. *Angew. Chem. Int. Ed* **9:610-9, 1970.** [Institutionen för organisk kemi, Kungl. Universitetet i Stockholm, Stockholm Va, Sandåsgatan, Sweden]

On methylation analysis of polysaccharides, a mixture of partially methylated sugars is obtained. Qualitative and quantitative analysis of this mixture gives valuable structural information. By performing this analysis by GLC-ms of the derived alditol acetates, this method became more accurate, faster, and required much less material. [The SC® indicates that this paper has been cited in more than 765 publications.]

Structural Analysis of Polysaccharides

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When, in 1966, I took over the chair in organic chemistry at Stockholm University, I decided to take up studies on the structure of immunologically active bacterial polysaccharides. One of the most important methods in such studies was the methylation analysis, introduced by Haworth and coworkers in the 1930s. It involves complete methylation, hydrolysis with acid, and qualitative and quantitative analysis of the partially methylated sugars formed. The methyl groups in these mark the nonsubstituted positions in the polysaccharide. The methylation reaction had been considerably improved by S. Hakomori,¹ but the analysis of the methylated sugars was time-consuming and minor components were easily overlooked. The analysis also required substantial amounts of polysaccharide, which was a drawback, in particular when

working with expensive biological material. To cover even a small part of this vast field, we had to improve the methods.

Commercial instruments for gas-liquid chromatography—mass spectroscopy (GLC-ms) had recently become available. There was only one such instrument at the university, in the Department of Analytical Chemistry; and we could use it only at night. I started the work with two graduate students, Hakan Björndal and Sigge Svensson, and we soon found that the alditol acetates prepared from the methylated sugars were ideal for our purpose. They were volatile, readily separated by GLC,² and gave typical, easily interpreted mass spectra.³ We could also do a complete analysis with less than 0.5 mg of polysaccharide.

Another graduate student, Calle G. Hellerqvist, had joined our group and started to investigate O-antigen polysaccharides from *Salmonella*. The leading scientists in this field were Otto Luderitz and Otto Westphal in Freiburg, Germany, and we could, with the new technique, correct some of their structures. When these results were published, I was invited to give a lecture at their institute. Westphal also arranged an invitation for me to write an article on our method for *Angewandte Chemie—international Edition in English*, which contributed to making the method known.

Even if there are now some alternative methods for methylation analysis of carbohydrates, our method is by far the most used. It has become so accepted that scientists generally do not refer to our work, except in review articles (as in reference 4).

1. **Hakomori S.** A rapid perraehtylation of glycolipid, and polysaccharide catalysed by methyl sulfinyl carbanion. *J. Biochem.* 55:205-8, 1964. (Cited 3,525 times.) [See also: **Hakomori S.** Citation Classic. *Current Contents/Life Sciences* 23(23): 12, 9 June 1980. Reprinted in: *Contemporary classics in the life sciences. Volume 2: the molecules of life.* (Barrett J T, ed.) Philadelphia: ISI Press, 1986. p. 57.]
2. **Björndal H, Lindberg B & Svensson S.** Gas-liquid chromatography of partially methylated alditols as their acetates. *Acta Chem. Scand.* 21:1801-4, 1967. (Cited 370 times.)
3. ----- Mass spectrometry of partially methylated alditol acetates. *Carbohydr. Res.* 5:433-40, 1967. (Cited 510 times.) [See also: **Lindberg B.** Citation Classic. *Current Contents/Life Sciences* 22(44): 10, 29 October 1979. Reprinted in: *Contemporary classics in the life sciences. Volume 2: the molecules of life.* (Barrett J T, ed.) Philadelphia: ISI Press, 1986. p. 207] 4. **Aspinall G O.** Chemical characterization and structure determination of polysaccharides. (Aspinall G O, ed.) *The polysaccharides.* New York: Academic Press, 1982. Vol. 1. p. 35-131.

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