MASS SPECTROMETRIC SEQUENCE ANALYSIS OF COMPLEX OLIGOSACCHARIDES
Comparison of the permethyl- and pertrimethylsilyl-derivatives of lacto-*N*-tetraose

Heinz EGGE, Hubertus v. NICOLAI and Fritz ZILLIKEN
Department of Physiological Chemistry, University Bonn, GFR

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1. Introduction

Complex oligosaccharides bound either to ceramides or to protein chains are integral components of cell surfaces [1–3]. Evidence has been presented by several laboratories that these oligosaccharide chains strongly influence the 'social behaviour' of cells like cell contact, contact inhibition of growth and movement, surface charge, immunological changes, antigenicity, agglutinability and viral and tumorigenic transformation [2–4]. It is obvious that there exists an increasing demand for structural elucidation of these carbohydrate chains.

Among the tedious and material consuming methods for structural characterization like consecutive enzymatic degradation [5] periodate degration [6] and permethylation [7, 8] mass spectrometry has proved to be a most powerful and elegant tool for microscale preparations.

Mass spectrometric data have been presented for permethyl [9, 10] pertrimethylsilyl [11] peracetyl [12] and cyclic boronic acid derivatives [13–15] of mono- and oligosaccharides as well as glycolipids. The data concerning complex oligosaccharides are still sparse and there exists still some controversy about optimal derivatization methods [10]. In order to shed some light on this question, the mass spectra of β-methyl-dodeca-*O*-methyl-lacto-*N*-tetraose and trimethylsilyl-LNT are discussed in the following paper. Butylboronic acid leading to a mixture of derivatives was not included.

2. Experimental

All solvents were of reagent grade, redistilled and dried before use. MSTFA (trifluormonotrimethylsilylacetamide) was obtained from Serva Heidelberg.

LNT from human milk was prepared by conventional methods, $[\alpha]_D = +25.5°$ (H_2O) [16].

One mg LNT dissolved in 10 μl DMF was treated with 20 μl MSTFA and 1 μl trimethylchlorosilan at room temperature. Aliquots containing 50 μg were transferred to the DI-probe vessel, carefully dried over KOH/P_2O_5 by gradual evacuation to 10^{-4} torr before introduction into the DI-probe inlet.

Permethylation and crystallization of β-methyl-dodeca-*O*-methyl-LNT has been published [17].

mp: 177–178°C $[\alpha]_D^{23} = +13°$ (c = 1 in $CHCl_3$)

$C_{39}H_{71}NO_{21}$ (889.9) calculated C: 52.63 H: 8.04 OCH_3: 45,33
found C: 52.37 H: 7.65 OCH_3: 45,87

Mass spectra were obtained on a LKB 9000 instrument.

Ionization energy 20 eV; trap current 60 μA; acc. Volt. 3.5 kV; ion source temp.: 250°C; sample evap. temp.: 20–150°C.

3. Results and discussion

The mass spectra of the pertrimethylsilyl and permethyl LNT are shown in fig. 1. The sample temperatures were 120° and 40°C respectively. The molecular ion of pertrimethylsilyl-LNT, M = 1643, is beyond the mass range of the instrument. Nevertheless all necessary fragments for the interpretation of the structure are present (fig. 2). Base peak is *m/e* 798 corresponding

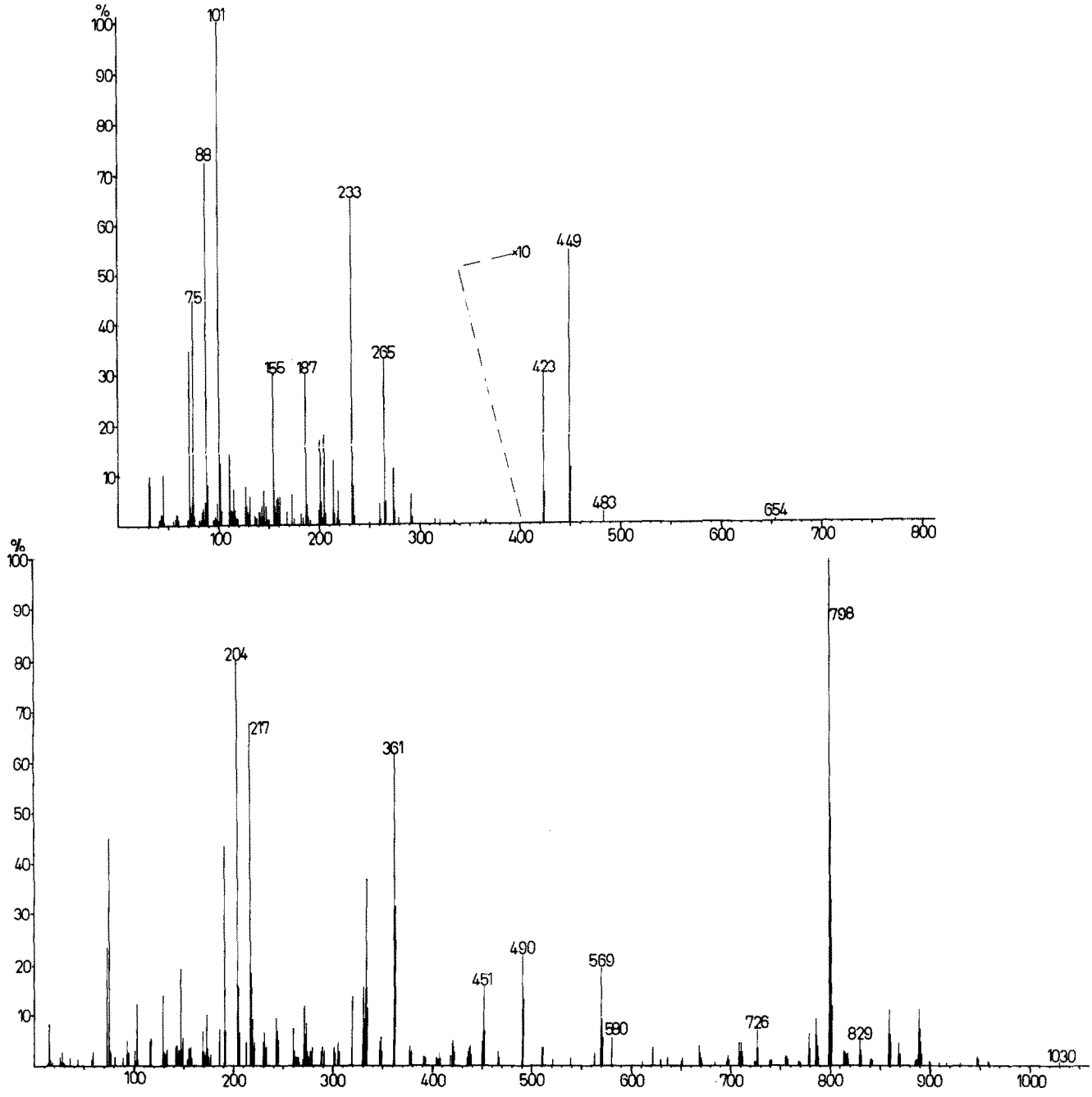

Fig. 1. Mass spectra of permethyl- and pertrimethylsilyl-lacto-*N*-tetraose.

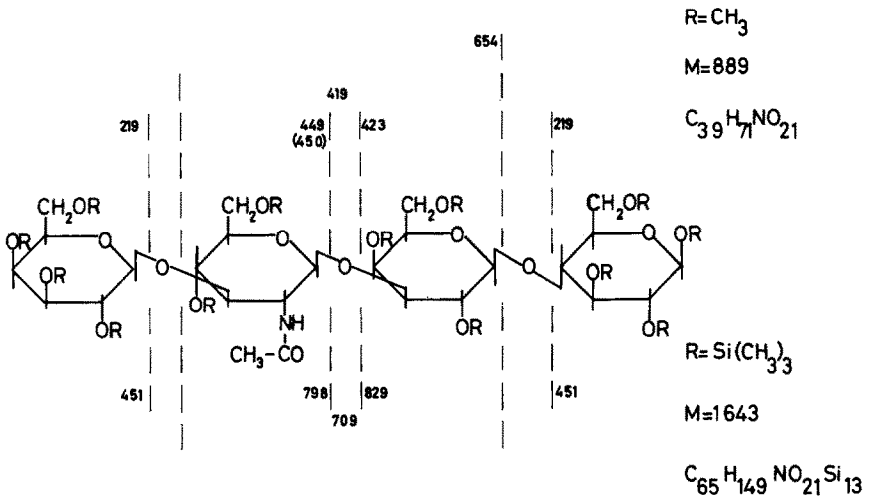

Fig. 2. Scheme of fragmentation.

to the lacto-N-biose (LNB) unit of the sugar. The lactose part is represented by *m/e* 829. The intensity of *m/e* 451 and 451-HOTMS = 361 as well as the absence of *m/e* 420 (tri-O-TMS-N-Ac-hexosaminyl-) indicates two hexoses at the reducing and nonreducing end of the molecule. From the very low intensity of *m/e* 173 (TMS-O-CH-NHAc), usually the base peak in 3-OTMS-hexosamines, it can be deduced, that the hexose is attached to position 3 of the hexosamine. Among the fragments derived from the hexose at the reducing end only *m/e* 668 is of diagnostic value being characteristic for (1–2) or (1–4)-bonds. Indirect evidence favors the (1–4) bond between the two hexoses. The high intensity of *m/e* 204 makes it likely, that more than one C_2–C_3-glycol group is present in the molecule.

For the bond between the two disaccharides two series of fragments arise from the degradation of the hexosamine or the adjoining galactose. The TMSO–CH = O-lactose is represented by *m/e* 947. This fragment containing C_1 of the hexosamine is of little diagnostic value for the type of bond as well as *m/e* 569 (TMSO–CH = O-hexose) derived from the lactose moiety.

The fragments *m/e* 1030; 580; 490 should arise from lacto-*N*-biosyl–O–C(=CH–OTMS)–CH = OTMS by subsequent loss of one hexose (450) and HOTMS. These fragments are common to (1–2), (1–3), or (1–4) bonds. The absence of a similar series 15 mass units smaller usually present in (1–2) and (1–4) bonds favors a (1–3) bond.

Base peak of permethyl LNT is *m/e* 101. In the higher mass range *m/e* 449 and 423 indicate the *N*-biose I and lactose halves of the sugar, the triose unit yields *m/e* 654. The hexoses at both ends of the molecule give rise to *m/e* 219. No *m/e* 246 is detectable excluding a terminal hexosamine. The LNB II [16] yields *m/e* 419 and 435. The low *m/e* 115 indicates a 3-*O*-glycosidic bond in the hexosamine moiety. In 3.4-, 3.6-di-*O*-methyl-*N*-acetyl-hexosamines *m/e* 115 is the most prominent fragment as in the 3,4,6-tri-*O*-methyl-derivatives. The occurrence of a (1–4) bond at the reducing end of the molecule is shown by *m/e* 279 and 161 [9]. Absence of *m/e* 380 excludes the fragment CHOMe–CHOMe–CHOMe–CH–O–Glc while *m/e* 291 may be an indication for a (1–3) bond of the *N*-biose to the lactose. In contrast to the results published on *N*-Methyl, *N*-ethyl-G_{m1} [10] the LNB I unit is represented by *m/e* 449 instead of 450. It could be shown however that the ratio 449/450 as well as the intensity of other important fragment ions is dependent on the sample temperature.

Comparing the results obtained, the permethyl derivative offers the advantage of small molecular weight, higher volatility and solvolytic stability. Furthermore after hydrolysis the partially methylated sugars can be qualitatively and quantitatively determined by GC–MS analysis either as alditol acetates [18] or methylglycosides. The amount of pure substance however, needed for this type of analysis will always be a severe handicap.

In spite of its high molecular weight, the pertrimethylsilyl derivative furnishes relatively low molecular fragments informative of important molecular features. The ease of preparation opens a wide field for structural investigations. Partial hydrolysis or acetolysis together with computerised GC–MS analysis of the OTMS derivatives of tri-, tetra- and pentasaccharides on high temperature GLC phases may allow a new structural approach to oligo- and polysaccharides on the μg to ng scale.

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