BIOCHIMIE — Extrait du Tome 60, n° 8, 1978, p. 725.

Printed in France.

Structure of the three major fucosyl-glycoasparagines accumulating in the urine of a patient with fucosidosis.

Gérard STRECKER ◇, Bernard FOURNET and Jean MONTREUIL.

Laboratoire de Chimie Biologique de la Faculté des Sciences et Techniques de Lille I et Laboratoire Associé au CNRS N° 217, Université des Sciences et Techniques de Lille I, B.P. n° 36, 59650 Villeneuve d'Ascq, France.

Lambertus DORLAND, Johan HAVERKAMP and Johannes F. G. VLIEGENTHART.

Organisch-Chemisch Laboratorium, Rijksuniversiteit Utrecht, Croesestraat 79, Postbus 5055, Utrecht, The Netherlands.

Dominique DUBESSET.

Service de Pédiatrie, Centre Hospitalier, 60200 Compiègne, France.

(*17-3-1978*).

Résumé.

Quinze fucosyl-glycoasparagines et fucosyl-oligosaccharides ont été isolés de l'urine d'un patient atteint de fucosidose, maladie liée à un déficit primaire en fucosidase lysosomiale.

Les structures des trois glycoasparagines majeurs sont les suivantes : α-Fuc-(1 → 6)-β-GlcNAc-Asn ; α-Man-(1 → 6)-β-Man-(1 → 4)-β-GlcNAc-(1 → 4) [α-Fuc-(1 → 6)]-β-GlcNAc-Asn ; β-Gal-(1 → 4) [α-Fuc-(1 → 3)]-β-GlcNAc-(1 → 2)-α-Man-(1 → 6)-β-Man-(1 → 4)-β-GlcNAc-(1 → 4) [α-Fuc-(1 → 6)]-β-GlcNAc-Asn.

Ces structures dérivent de la classe des fucosyl-glycoprotéines (ex : IgG immunoglobulines, lactotransferrine et α_1-glycoprotéine acide).

La séquence terminale : β-Gal-(1 → 4) [α-Fuc-(1 → 3)] β-GlcNAc-(1 → 2)-α-Man → R est un nouveau type de structure présent dans des fractions glycanniques de glycoprotéines.

Summary.

Fifteen fucosyl-oligosaccharides and fucosyl-glycoasparagines have been isolated from the urine of a patient with fucosidosis. The structure of the three most abundant glycoasparagines are as follows :

α-Fuc-(1 → 6)-β-GlcNAc-Asn ; α-Man-(1 → 6)-β-Man-(1 → 4)-β-GlcNAc-(1 → 4) [α-Fuc-(1 → 6)]-β-GlcNAc-Asn ; β-Gal-(1 → 4) [α-Fuc-(1 → 3)] β-GlcNAc-(1 → 2)-α-Man-(1 → 6)-β-Man-(1 → 4)-β-GlcNAc-(1 → 4) [α-Fuc-(1 → 6)] β-GlcNAc-Asn.

The structures are related to the class of fucosyl-glycoproteins (e.g.: IgG immunoglobulin, lactotransferrin and α_1-acid glycoprotein).

The terminal sequence : β-Gal-(1 → 4) [α-Fuc-(1 → 3)] β-GlcNAc-(1 → 2)-α-Man → R is novel for carbohydrate moieties in glycoproteins.

◇ *To whom all correspondence should be addressed.*

List of Abbreviations :

Fuc : L-fucose.
GlcNAc : N-acetyl-D-glucosamine.
GalNAc : N-acetyl-D-galactosamine.
Gal : D-galactose.
Man : D-mannose.
Asn : L-asparagine.
MHz : megahertz.
^{1}H-NMR : Proton nuclear magnetic resonance.
ppm : parts per million.
δ : chemical shift.
m/e : mass/charge ratio.
$J_{1,2}$: coupling constant (in Hz) of C-1 and C-2 protons.

Introduction.

Fucosidosis is an inborn error of metabolism involving an α-L-fucosidase defect leading to the accumulation of fucosyl-glycoconjugates in tissues and body fluids [1, 2]. Tsay *et al.* characterized three of these compounds in brain, cultured fibroblasts and urine [3, 4]. Six other oligosaccharides and glycoasparagines have recently been isolated from the urine of two patients [5]. Here, we describe the isolation of 15 fucosyl-oligosaccharides and fucosyl-glycoasparagines from the urine of a third patient whereas the structure elucidation of the three major glycoasparagines by permethylation analysis and 360 MHz ^{1}H-NMR spectroscopy is detailed.

Materials and Methods.

Fractionation of urinary fucosides.

1 l of freshly collected urine from a patient with A_2B, Le^b phenotype was fractionated by ion exchange and charcoal-Celite chromatography as described previously [6]. Oligosaccharides and glycoasparagines were purified by paper chromatography using the solvent system : pyridine/ethyl acetate/acetic acid/water (5:5:1:3). Chromatograms were stained with aniline oxalate reagent [7] or with 1 per cent ninhydrine in acetone.

Analytical methods.

The molar ratios of neutral monosaccharides and hexosamines were determined after methanolysis (0.5 N methanolic HCl, 80°C during 24 h) [8]. Molar ratios of glucosamine and aspartic acid were determined on a Beckman amino-acid analyser, after hydrolysis in 4N CF_3COOH for 4 h at 100°C.

Structural methods.

Defucosidation was carried out by partial acid hydrolysis in 0.05 N H_2SO_4 for 20 min at 100°C. The reducing terminal monosaccharide residue was identified as a polyol, after reduction with sodium borohydride. Methylation of glycoasparagines was performed according to Hakomori [9]. *O*-methyl ethers and partially *O*-acetylated-*O*-methyl ethers were identified as described by Fournet *et al.* [10]. Hydrazinolysis-nitrous deamination was carried out according to Bayard *et al.* [11]. The reduced 2,5-anhydromannitol-containing oligosaccharides were methylated [9] and separated on a column of 3 per cent SE-30 operated with a temperature gradient of 6°C/min from 200°C to 290°C. They were detected by total ionization current and mass fragmentography at m/e values 189 and 219 (Riber-Mag 10-10 apparatus Rueil-Malmaison, France).

Proton exchange : the glycoasparagines were dissolved in deuterium oxide and after standing at room temperature for 6 h lyophilized. This procedure was repeated five time to get a high degree of proton exchange.

The 360 MHz ^{1}H-NMR spectra were recorded on a Brucker HX-360 spectrometer, operating in the Fourier transform mode at probe temperatures of 25°C or 60°C. Chemical shifts are given relative to sodium 2,2-dimethyl-2-silapentane-5-sulphonate (indirectly to acetone in D_2O : $\delta = 2.225$ ppm).

FIG. 1. — *Paper chromatography of fucosyl-glycoasparagines eluted from a cation exchanger* (Dowex 50 × 2 ; 200-400 mesh ; H⁺) *by a discontinuous gradient of pyridine-acetate buffer* (*pH 5.4*) : 1 : 1 mM ; 2 : 2 mM ; 3 : 5 mM ; 4 : 10 mM ; 5 : 20 mM ; 6 : 50 mM ; 7 : 100 mM. Solvent : pyridine/ethyl acetate/acetic acid/water (5:5:1:3). Time of migration : 18 h.

Results.

FRACTIONATION OF GLYCOASPARAGINES AND OLIGOSACCHARIDES FROM URINE.

Accumulated fucose-containing material was isolated as two fractions : 1) material not retained on ion exchangers, 2) material eluted from Dowex 50 × 2 by pyridine-acetate buffers. Glycoasparagines 1 and 2 were eluted by 5 and 2 mM pyridine-acetate buffer respectively, whereas glycoasparagines 3 to 8 were found in the 1 mM fraction (fig. 1). These products were purified by paper preparative chromatography (fig. 2). Since fraction 1 was too complex for further purification, it was fractionated on charcoal-Celite. The oligosaccharide OL_1 to OL_5 and the glycoasparagine GP_9 were eluted by a discontinuous gradient of ethanol (Fig. 3).

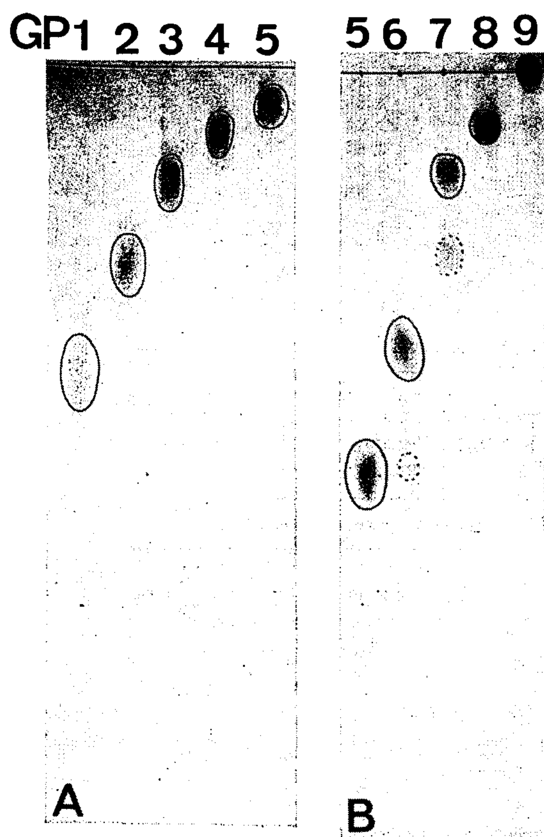


FIG. 2. — *Paper chromatography of isolated glycoasparagines.* Solvent : see figure 1. Time of migration : A : 3 days, B : 20 days.

The glycoasparagines GP_1, GP_3, GP_8 and GP_9 are present in relatively large amounts (table I).

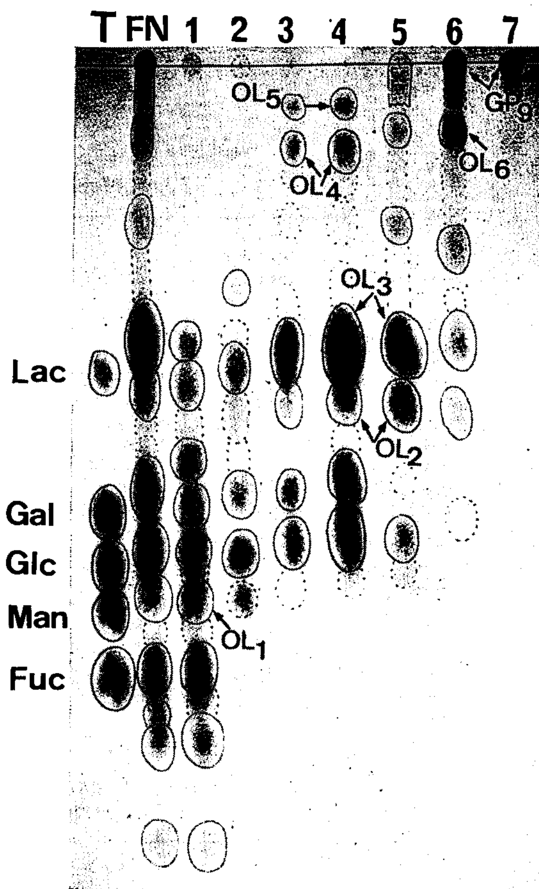


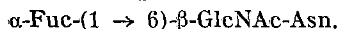
FIG. 3. — *Paper chromatography of neutral oligosaccharide eluted from a charcoal-Celite column by a discontinuous gradient of ethanol.* F_N : neutral fraction before charcoal-Celite chromatography. Concentrations of ethanol : 1 : 1.5 % ; 2 : 3.5 % ; 3 : 5 % ; 4 : 7.5 % ; 5 : 10 % ; 6 : 15 % ; 7 : 50 %. Solvent : see figure 1. Time of migration : 18 h.

STRUCTURE OF GLYCOASPARAGINES GP_1, GP_3 AND GP_8.

The molar carbohydrate composition of the glycoasparagines GP_1, GP_3 and GP_8 are reported in table I. The results of methylation analysis are given in table II and figure 4.

The detailed 360 MHz ^{1}H-NMR data for GP_1 have been published previously [12]. For the determination of the position of the fucosyl residue in this compound also ^{13}C-NMR data were used [12]. On

the basis of the chemical and NMR results (table III) GP_1 was identified as :


α-Fuc-(1 → 6)-β-GlcNAc-Asn.

After defucosidation of GP_3 by partial acid hydrolysis, only 3,6-di-*O*-methyl-*N*-acetylglucosamine was found proving the (1 → 6) linkage of fucose to *N*-acetylglucosamine. Hydrazinolysis - nitrous deamination, followed by KBH_4 reduction gave a mixture of two components, one composed of mannose and 2,5 anhydro-mannitol (2 : 1) the other composed of fucose and 2,5 anhydro-mannitol (1 : 1).

The 360 MHz spectrum of glycoasparagine GP_3 is given in figure 5. The anomeric region of the spectrum (4.4 - 5.2 ppm) contains five well resolved proton signals of equal intensity, indicating that the total number of sugar monomers in this glycoasparagine is five. The two signals with

TABLE I.

Characteristics of urinary fucosides.

	Quantity (mg/l)	Molar ratios						
		Fuc	Gal	Man	Glc	GlcNAc	GalNAc	Asn
GP_1	85	0.89				0.97		1
GP_2	4	0.85	0.88			0.96		1
GP_3	29	0.95		2.05		2.08		1
GP_4	3	0.89	0.92	1.92		2.87		1
GP_5	2.5	0.91	0.94	1.94		2.80		1
GP_6	4	1.94	1.05	1.89		2.94		1
GP_7	5	1.91	1.07	1.80		2.87		1
GP_8	22	1.89	1.02	2.07		2.91		1
GP_9	112	4.87	3.95	3.05		5.85		1
OL_1	5	0.89				1		
OL_2	16	1.04	1.02				1	
OL_3	95	1.02	2					
OL_4	22	1.97	1.92		1			
OL_5	8	2.95	3.10			1		
OL_6	21	0.98	1.12	1.98		2		

TABLE II.

Molar ratios of monosaccharides methyl ethers present in the permethylated glycoasparagines.

Monosaccharide methyl ethers	Molar ratios in glycoasparagines				
	GP_1	GP_3	GP_3 minus fucose	GP_8	GP_8 minus fucose
Methyl 2,3,4-tri-*O*-methyl fucoside	+	1.07		2.16	
Methyl 2,3,4,6-tetra-*O*-methyl galactoside				0.87	1.02
Methyl 2,3,4,6-tetra-*O*-methyl mannoside		1.05	1.02		
Methyl 2,3,4-tri-*O*-methyl mannoside		1	1	1	1
Methyl 3,4,6-tri-*O*-methyl mannoside				0.88	0.91
Methyl 3,4-di-*O*-methyl-*N*(acetyl) methyl-glucosaminide	+				
Methyl 3,6-di-*O*-methyl-*N*(acetyl) methyl glucosaminide		+	+	+	+
Methyl 6-mono-*O*-methyl-*N* (acetyl) methyl glucosaminide				+	
Methyl 3-mono-*O*-methyl-*N* (acetyl) methyl glucosaminide		+		+	

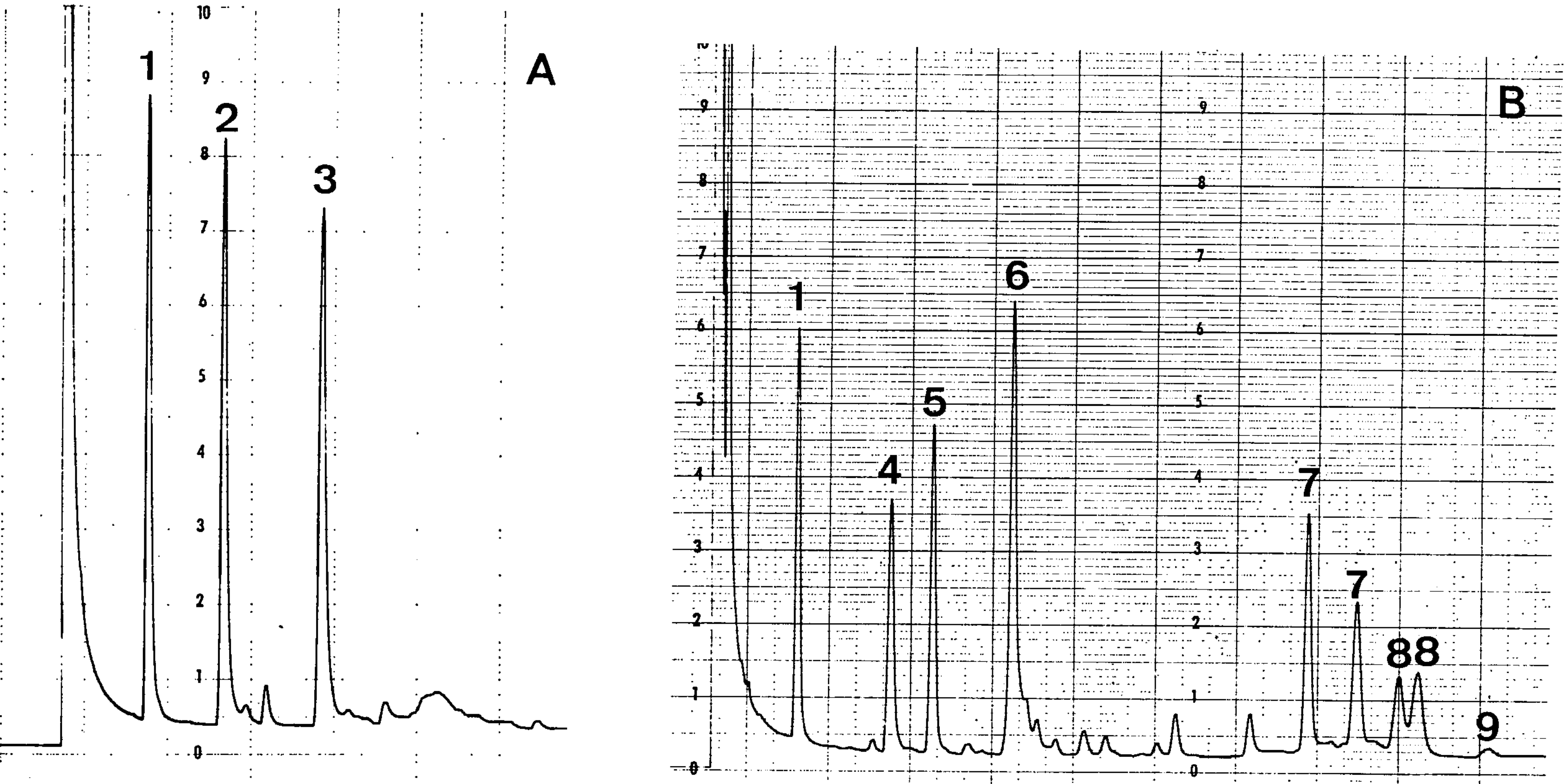


FIG. 4. — *Identification of methyl ether glycosides obtained by methanolysis of permethylated glycoasparagines.* A : GP_3 (methyl ether glycosides) ; B : GP_8 (acetylated methyl ether glycosides). 1 : methyl 2,3,4-tri-*O*-methyl fucoside ; 2. methyl 2,3,4,6-tetra-*O*-methyl mannoside ; 3 : methyl 2,3,4-tri-*O*-methyl mannoside ; 4 : methyl 2,3,4,6-tetra-*O*-methyl galactoside ; 5 : methyl 3,4,6-tri-*O*-methyl-2-mono-*O*-acetyl mannoside ; 6 : methyl 2,3,4-tri-*O*-methyl-6-mono-*O*-acetyl mannoside ; 7 : methyl 3,6-di-*O*-methyl-*N*(methyl)acetyl-4-mono-*O*-acetyl-glucosaminide ; 8 : methyl 6-mono-*O*-methyl-*N*(methyl)acetyl-3,4-di-*O*-acetyl glucosaminide ; 9 : methyl 3-mono-*O*-methyl-*N*(methyl)acetyl-4,6-di-*O*-acetyl glucosaminide. Aerograph 1 200 apparatus. Glass column (0.2 × 300 cm). Stationary phase : Chromosorb W-HMDS, with 3 per cent Carbowax 6 000 ; Gas carrier, N_2 ; 30 ml/min. Temperature : 110°C to 200°C (2°C/min).

TABLE

¹H-NMR data (a) of anomeric protons, mannose-H_2 protons,

Compounds

Compounds
1 β-GlcNAc-Asn α-Fuc-(1 → 6) 7
3 2 1 β-Man-(1 → 4)-β-GlcNAc-(1 → 4)-β-GlcNAc-Asn 4 α-Man-(1 → 6) α-Fuc-(1 → 6) 7
3 2 1 β - Man - (1 → 4) - β - GlcNAc - (1 → 4) - β - GlcNAc - Asn 6 5 4 β - Gal - (1 → 4) - β - GlcNAc - (1 → 2) - α - Man - (1 → 6) α-Fuc-(1 → 3) 8 α-Fuc-(1 → 6) 7

(a) Chemical-shifts δ are given in ppm downfield from sodium 2-2-dimethyl-2-silapentane-5-sulphonate ; the values in brackets

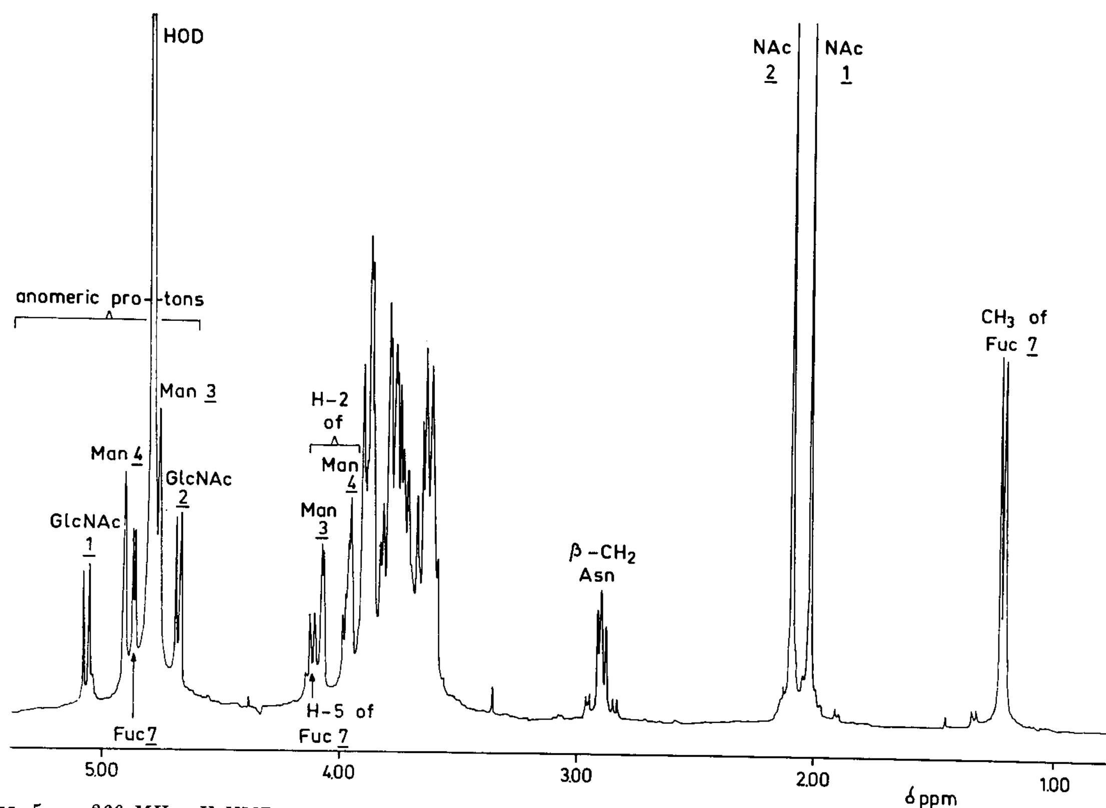


FIG. 5. — *360 MHz ¹H NMR spectrum of glycoasparagine GP₃, isolated from the urine of a patient with fucosidosis.*

III.

N-*acetyl methyl protons and fucose-methyl protons.*

H-1 of residue								H-2 of residue		NAc of residue			CH_3 of residue		H-5 of residue	
1	2	3	4	5	6	7	8	3	4	1	2	5	7	8	7	8
5.09 (9.8)	—	—	—	—	—	4.90 (3.7)	—	—	—	2.02	—	—	1.21	—	4.12	—
5.07 (9.5)	4.69 (7.0)	4.77 (~1)	4.92 (1.2)	—	—	4.88 (3.7)	—	4.08	3.96	2.02	2.09	—	1.21	—	4.12	—
5.07 (9.4)	4.69 (7.5)	4 77 (~1)	4.91 (~1)	4.58 (7.5)	4.45 (7.5)	4.88 (3.8)	—	4.09	4.09	2.02	2.10	2.04	1.22	1.19	4.13	4.83

are coupling constants $J_{1,2}$ in Hz.

$J_{1,2} \sim 1$ Hz represent the two mannose residues, the signal with $J_{1,2} = 3.9$ Hz the α-fucose residue and the two signals with $J_{1,2} = 6.8$ and 9.5 Hz respectively stem from the β-GlcNAc residues. At about $\delta = 2$ ppm two N-acetyl methyl signals (2×3 protons) are found in a 1 : 1 ratio. The doublet at $\delta = 1.21$ ppm (3 protons) stems from the CH_3 group of the fucose residue. The H-5 proton of the fucose residue resonates at $\delta = 4.12$ ppm.

The resonance positions of the H-1 proton of the mannose residues at $\delta = 4.92$ and $\delta = 4.77$ ppm give direct information about the mannose-mannose glycosidic linkage. As has been discussed in more detail recently [13] the presence of the signal at $\delta = 4.92$ together with the absence of a signal at $\delta = 5.12$ indicate that the sequence α-Man-(1 → 6)-β-Man-(1 → 4)-GlcNAc occurs. From the position of the mannose H-2 resonance at $\delta =$ 3.96 ppm it can be concluded that mannose residue 4 is not glycosylated in position C_2 [13]. It has to be noted that the fucose residue, α-(1 → 6) linked to GlcNAc residue 1 gives rise to a down field shift of H_1 of GlcNAc residue 2 (4.61 → 4.69 ppm).

The NMR-data of GP_3 are summarized in table III. On the basis of the chemical and NMR analysis GP_3 was established to be : α-Man-(1→6)-β-Man-(1 → 4)-β-GlcNAc-(1 → 4) [α-Fuc-(1 → 6)] β-GlcNAc-Asn.

After defucosidation of GP_8, only 3,6-di-*O*-methyl-*N*-acetylglucosamine was found. This indicates that one fucose residue is (1 → 6) linked to a GlcNAc residue, whereas the other fucose is linked (1 → 3) to another GlcNAc residue. Hydrazinolysis-nitrous deamination followed by reduction gave a mixture of three components with the following compositions : fucose and 2,5 anhydromannitol (1 : 1) ; mannose and 2,5 anhydro-mannitol (2 : 1) ; galactose, fucose and 2,5 anhydromannitol (1 : 1 : 1). The mass-spectra of these reduced-methylated compounds are given in figure 6. Peak 2 gave the fragments aA_1 (m/e 219) and cA_1 (m/e 189) which are significant for terminal hexose and 6-deoxy-hexose. This branched structure was confirmed by the occurence of fragments $cbaJ_1$ (m/e 423) and $abcJ_1$ (m/e 453). The major fragments obtained from peaks 1 and 3 are given in figure 6. These demonstrate that in GP_8 the following structure units occur : Fuc-GlcNAc ; Man-Man-GlcNAc ; Fuc[Gal]GlcNAc.

Relevant 360 MHz ^{1}H-NMR data of GP_8 are summarized in table III. The anomeric region of the spectrum contains two signals (1 proton each) with $J \simeq 1$ Hz, representing the H-1 atoms of the mannose residues, four doublets with $J = 7.5$ -

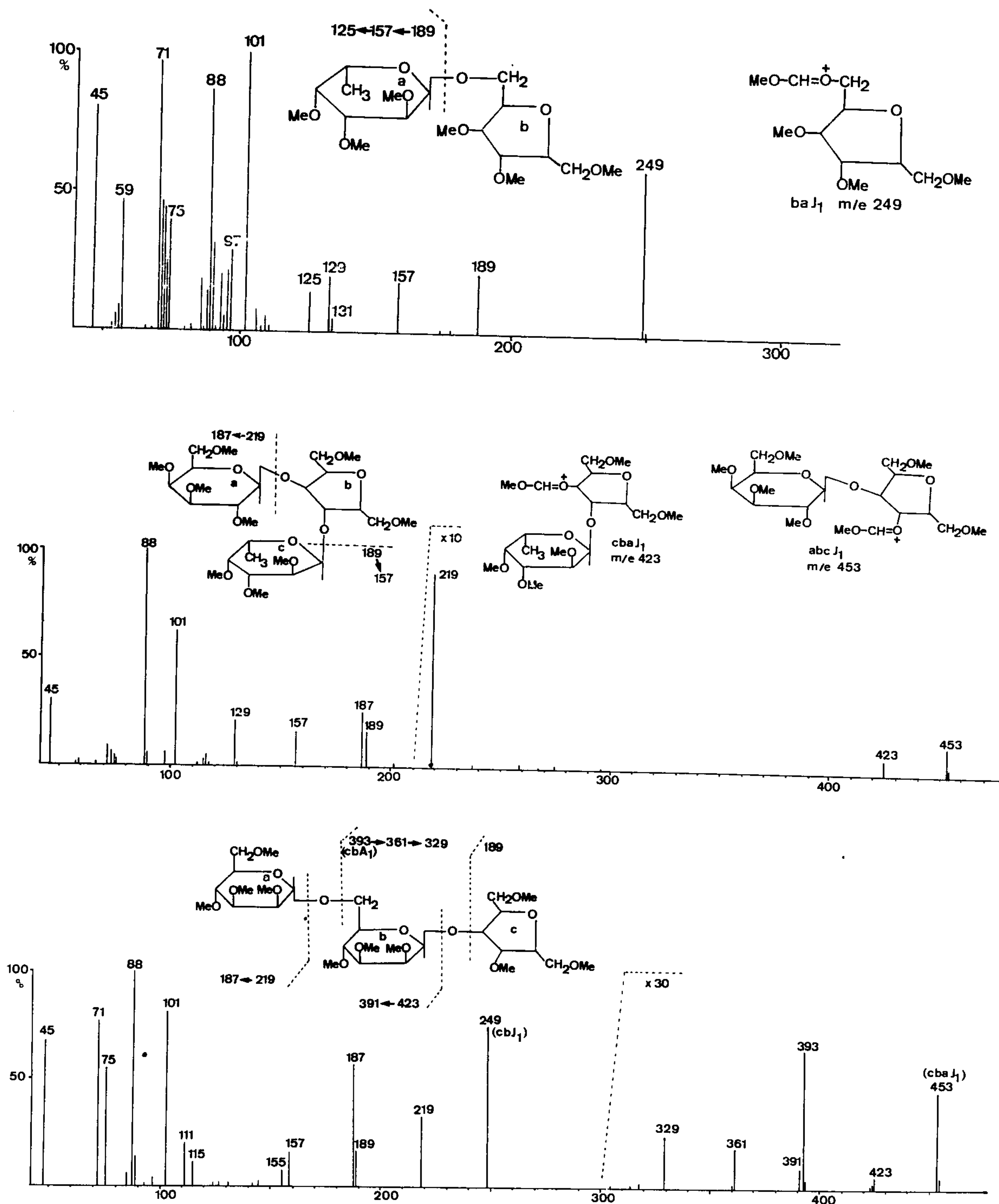


FIG. 6. — *Mass spectrometry of methylated oligosaccharides obtained by hydrazinolysis-nitrous deamination and separated by gas-liquid chromatography* (see text).

9.4 Hz, representing the H-1 atoms of the β-linked galactose and N-acetylglucosamine residues and two doublets with J = 3.8 and 4.0 Hz for the H-1 atoms of the two α-L-fucose residues.

The similarity of the H-1 data from residues 1-4 and 7 with those of GP-3 together with the presence of three additional H-1 proton signals indicate that the structure of GP-8 is an extended form of that of GP-3.

The structure of the extra trisaccharide unit β-Gal-(1 → 4)-[α-Fuc-(1 → 3)]-GlcNAc is expressed in the resonance positions of the fucose H-1, H-5

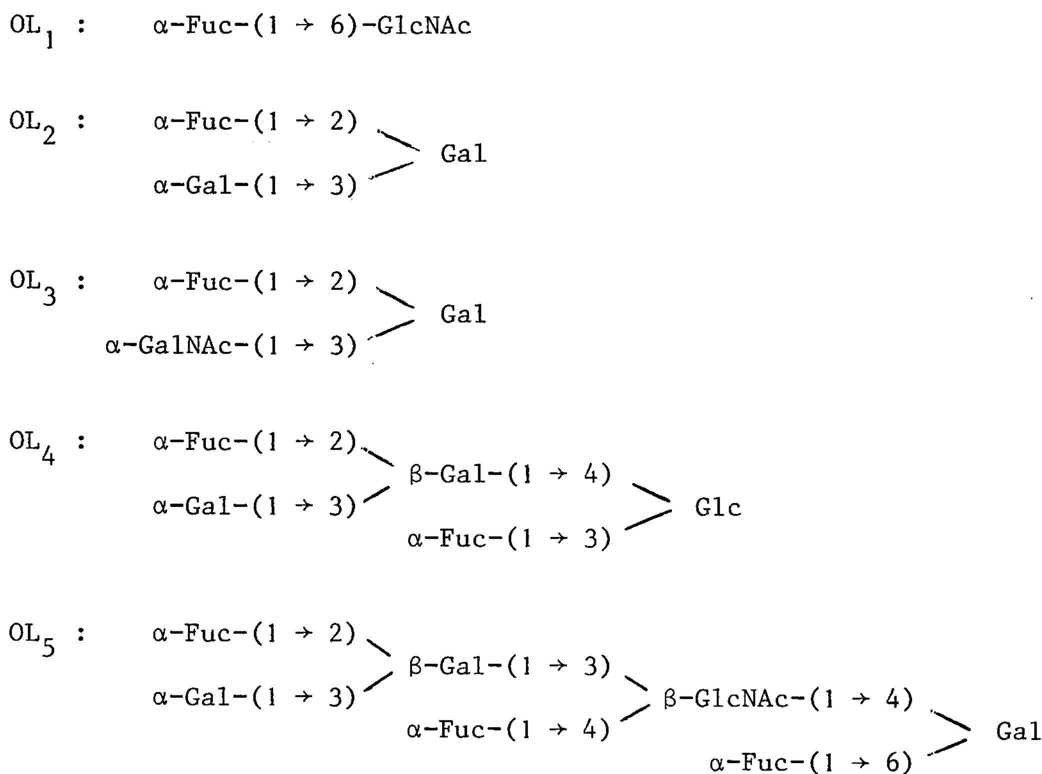


Fig. 7. — *Structures of oligosaccharides OL_1 to OL_5* [5, 17, 18].

and H-6 protons (5.13 ; 4.83 and 1.19 ppm respectively) [14], whereas the shift of the H-2 of mannose residue 4 from 3.96 (in GP_3) → 4.09 ppm indicates that the trisaccharide unit is linked to C_2 of this mannose residue [13]. It is interesting to note that the two different terminal fucose residues can be distinguished on the basis of the NMR data.

On the basis of the chemical and NMR analysis GP_8 was established to be : β-Gal-(1 → 4) [α-Fuc-(1 → 3)] β-GlcNAc-(1 → 2)-α-Man-(1 → 6)-β-Man-(1 → 4)-β-GlcNAc-(1 → 4) [α-Fuc-(1 → 6)] β-GlcNAc-Asn.

Discussion.

The structures of fucosyl-glycoasparagines accumulated in urine of fucosidosis patients are related to the glycan moieties of glycoproteins containing fucose. It is interesting to note that GP_8 possesses the terminal sequence β-Gal-(1 → 4) [α-Fuc-(1 → 3)] β-GlcNAc → R. This structural element is known to occur in milk oligosaccharides [15] but is novel for mannose containing glycoproteins. Recently, this trisaccharide unit was also discovered in α_1-acid glycoprotein [16].

It is also interesting to note that from the accumulated material which is related to glycoprotein catabolism (e.g. GP_1-GP_9, OL_1 and OL_6) 90 % are fucosyl-glycoasparagines whereas the fucosyl-oligosaccharides OL_1 and OL_6 are minor constituents. The decasaccharide

```
α-Fuc-(1 → 2)-β-Gal-(1 → 4)-β-GlcNAc-(1 → 2)-α-Man-(1 → 3)
                                                            \
                                                             β-Man-(1 → 4)-GlcNAc
                                                            /
α-Fuc-(1 → 2)-β-Gal-(1 → 4)-β-GlcNAc-(1 → 2)-α-Man-(1 → 6)
```

described by Tsay *et al.* [4] as the major constituent of fucosidosis urine (0.4 g/l) was absent in our samples [5]. According to the experimental procedure followed by these authors, it is possible that this dicasaccharide consisted of a mixture of several components.

The oligosaccharides OL_2 to OL_5 (Fig. 7), which have been previously characterized in normal urine or during induced galactosuria [17, 18] are known to be products of direct glycosylation of free galastose, glucose and lactose [17], and their increase in fucosidosis urine is associated with the fucosidase defect.

Acknowledgements.

We thank Mr. Y. Leroy, Mrs. A. Lemaire and A. F. Bouquelet for their skilful technical assistance. This investigation was supported in part by the Centre National de la Recherche Scientifique (Laboratoire Associé n° 217 : Biochimie physico-chimique et moléculaire des glucides libres et conjugués and RCP n° 425 : Glucides et Glycoconjugués), the Institut National de la Santé et de la Recherche Médicale (U. 124 : Unité de Recherches Ultrastructurales et Biochimiques sur les cellules normales et cancéreuses) the Fondation pour la Recherche Médicale Française and the Netherlands Foundation for Chemical Research (SON) *with financial aid from the Netherlands Organization for the Advancement of Pure Research* (ZWO). *Authors* (G.S., B.F., J.M.) *are grateful, to the Délégation Générale à la Recherche Scientifique et Technique, the Etablissement Public Régional Nord-Pas-de-Calais and Institut Pasteur of Lille for the contribution toward the purchase of the Riber Mass Spectrometer.*

REFERENCES.

1. Durand, P., Philippart, M., Borrone, C. & Della Cella, G. (1969) *Minerva Pediat.*, 84, 160-166.
2. Durand, P., Borrone, C. & Della Cella, G. (1969) *J. Pediat.*, 75, 665.
3. Tsay, G. C. & Dawson, G. (1976) *J. Neurol.*, 27, 733-740.
4. Tsay, G. C., Dawson, G. & Sung, J. (1976) *J. Biol. Chem.*, 251, 5852-5859.
5. Strecker, G., Fournet, B., Spik, G., Montreuil, J., Durand, P. & Tondeur, M. (1977) *C. R. Acad. Sci.* Paris, 284, 85-88.
6. Strecker, G. & Lemaire-Poitau, A. (1977) *J. Chromatog. Biomed. Applic.*, 143, 553-569.
7. Partridge, S. M. (1950) *Biochem. J. Symp.*, 3, 52-61.
8. Zanetta, J. P., Breckenridge, W. C. & Vincendon, G. (1972) *J. Chromatog.*, 69, 291-301.
9. Hakomori, S. (1964) *J. Biochem.*, 55, 205-208.
10. Fournet, B. & Montreuil, J. (1973) *J. Chromatog.*, 75, 29-37.
11. Bayard, B. & Montreuil, J. (1974) Colloq. Int. Cent. Natl. Rech. Sci. n° 221, 209-218, C.N.R.S., éd., Paris.
12. Dorland, L., Schut, B. L., Vliegenthart, J. F. G., Strecker, G., Fournet, B., Spik. G. & Montreuil, J. (1977) *Eur. J. Biochem.*, 73, 93-97.
13. Dorland, L., Haverkamp, J., Schut, B. L., Vliegenthart, J. F. G., Spik, G., Strecker, G., Fournet, B. & Montreuil, J. (1977) *FEBS Letters*, 77, 15-20.
14. Montreuil, J. & Vliegenthart, J. F. G. *Proc. Vth Intern. Symp. on Glycoconjugates*, Woods Hole (U.S.A.) Sept. 27-Oct. 1, 1977, Acad. Press, New York (in press).
15. Kobata, A. & Ginsburg, V. (1969) *J. Biol. Chem.*, 244, 5496-5499.
16. Fournet, B., Strecker, G., Spik, G., Montreuil, J., Dorland, L., Haverkamp, J., Schut, B. L. & Vliegenthart, J. F. G. *Proc. Vth Intern. Symp. on Glycoconjugates*, Woods Hole (U.S.A.) Sept 27 - Oct. 1, 1977, Academic Press, New York (in press).
17. Strecker, G., Trentesaux-Chauvet, C., Poitau, A. & Montreuil, J. (1976) *Biochimie*, 58, 805-814.
18. Strecker, G., Riazi-Farzad, T., Fournet, B., Bouquelet, S. & Montreuil, J. (1976) *Biochimie*, 58, 815-825.