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ISOLATION OF THE MAJOR O-GLYCOSIDICALLY LINKED OLIGOSACCHARIDES OBTAINED BY ALKALINE BOROHYDRIDE DEGRADATION OF HUMAN MECONIUM GLYCOPROTEINS

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SUMMARY

Neutral and acidic oligosaccharides derived from human meconium glycoproteins by alkaline borohydride degradation have been separated by high-performance liquid chromatography on a Micro-Pak anion-exchange column. In each class, oligosaccharides were purified by normal-phase (neutral and acidic oligosaccharides) and reversed-phase (neutral oligosaccharides) chromatography. Effective separations of neutral oligosaccharides and acidic oligosaccharides were achieved.

INTRODUCTION

The intestinal glycoproteins of human meconium are characterized as high-molecular-mass compounds possessing many hundred covalently attached oligosaccharide units, which express a number of differentiation and oncofoetal carbohydrate antigens [1–4]. Studies carried out by Hounsell et al. [5] have established the structures of nine major mono- to tetrasaccharides, O-glycosidically linked, from human meconium glycoproteins and from group O-secretors. The meconium glycoproteins treated by pronase were fractionated into Ii antigen enriched and depleted fractions. The latter fraction was subjected to mild acid hydrolysis to enhance the expression of the oncofoetal antigens. The asialo- and afucooligosaccharides were obtained, after base–borohydride degradation, by high-performance liquid chromatography (HPLC) on octadecylsilyl and aminopropylsilyl columns.

This paper reports the separation of oligosaccharide-alditols obtained by alkaline borohydride treatment of human meconium by different HPLC tech-

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niques: anion-exchange chromatography, partition chromatography on primary amine-bonded silica and reversed-phase chromatography.

EXPERIMENTAL

Glycoproteins and oligosaccharides

The new-born meconium was prepared as described previously [6]; 0.51 g of carbohydrate material was treated with 0.1 *M* potassium hydroxide–1 *M* potassium borohydride for 18 h at 45°C [7]. The resulting oligosaccharide-alditols were purified by gel permeation on a Bio-Gel P-6 (−400 mesh; Bio-Rad Labs., Richmond, CA, U.S.A.) column (85 cm × 2 cm I.D.) eluted with 0.5% acetic acid at a flow-rate of 18 ml/h. The oligosaccharides were detected at 206 nm (LKB 2138 Uvicord S, Bromma, Sweden).

Separation of oligosaccharide-alditols from Bio-Gel P-6 fraction of alkaline borohydride degradation of human meconium glycoproteins by HPLC on quaternary amine-bonded silica [8]

HPLC was performed on a 10-μm Micro-Pak AX-10 column (50 cm × 0.8 cm I.D.; Varian, Walnut Creek, CA, U.S.A.) with a Spectra-Physics Model 8700 liquid chromatograph (San Jose, CA, U.S.A.) equipped with an LDC variable-wavelength detector (Spectro Monitor D, Milton Roy, Riviera Beach, FL, U.S.A.) connected to a Spectra-Physics Model 4100 computing integrator.

For preparative chromatography, 48 mg of the Bio-Gel P-6 fraction (FII) were subjected to HPLC according to Paz-Parente et al. [9].

Each fraction, except the neutral oligosaccharide-alditols FII-N eluted with water, was purified by gel permeation on a Bio-Gel P-2 (200–400 mesh; Bio-Rad Labs.) column (92 cm × 2 cm I.D.) eluted with 0.5% acetic acid at a flow-rate of 15 ml/h. The fractions were revealed with orcinol–sulphuric acid reagent [10] on silica gel plates (pre-coated silica gel 60; Merck, Darmstadt, F.R.G.).

HPLC separation of neutral oligosaccharide-alditols on primary amine-bonded silica

HPLC was performed on a 5-μm Amino Zorbax column (25 cm × 0.94 cm I.D.; Du Pont de Nemours, Wilmington, DE, U.S.A.). A 11.5-mg amount of oligosaccharide-alditols FII-N dissolved in 100 μl of water was injected. The column was equilibrated with the initial solvent (acetonitrile–water, 70:30, v/v). After the injection, isocratic conditions were applied for 10 min with the initial solvent, followed by a linear gradient to acetonitrile–water (60:40, v/v) for 60 min and then isocratic conditions for 20 min. The flow-rate was 3 ml/min. The oligosaccharides were detected at 200 nm.

HPLC separation of oligosaccharide-alditols from primary amine-bonded silica HPLC fractions on an octadecylsilyl-bonded column

HPLC was performed on a 5-μm ODS Zorbax column (25 cm × 0.94 cm I.D.; Du Pont Instruments). Each fraction from primary amine-bonded silica HPLC was refractionated by reversed-phase chromatography. The column was equilibrated with distilled water. After injection, isocratic conditions were applied for

15 min with the initial solvent (water), followed by a linear gradient to water–acetonitrile (90:10, v/v) for 120 min. The flow-rate was 3 ml/min. The oligosaccharide-alditols were detected at 200 nm. The chart speed of the integrator was 0.5 cm/min.

HPLC separation of acidic oligosaccharide-alditols FII-1 and FII-2 on primary amine-bonded silica [11]

HPLC was performed on a 5-μm Amino Zorbax column (25 cm$\times$0.94 cm I.D.; Du Pont Instruments). The oligosaccharide-alditols FII-1 fraction (7.7 mg) dissolved in water (80 μl) was injected into the column. The column was equilibrated with the initial solvent (acetonitrile–15 m*M* potassium dihydrogenphosphate adjusted to pH 5.2; 75:25, v/v). After the injection, isocratic conditions were applied with the initial solvent for 25 min, followed by a linear gradient to acetonitrile–15 m*M* potassium dihydrogenphosphate (35:65, v/v) for 60 min and then isocratic conditions for 30 min. The flow-rate was 3 ml/min. The oligosaccharide-alditols were detected at 200 nm.

All the collected fractions were purified by gel permeation on a Fractogel TSK HW-40 column (25–40 μm, 36 cm$\times$1.6 cm I.D.; Merck) with a Shimadzu LC-5A liquid chromatograph equipped with an LKB Uvicord S 2138 detector and an SP 6040 differential refractometer. Elution was carried out with 0.5% acetic acid at a flow-rate of 1 ml/min.

Molar composition of oligosaccharide-alditols

The molar composition of oligosaccharide-alditols was determined by gas chromatography (GC) after methanolysis (0.5 *M* hydrochloric acid–methanol for 24 h at 80°C), N-reacetylation and trimethylsilylation according to Kamerling et al. [12] modified by Montreuil et al. [13].

RESULTS AND DISCUSSION

The separation of a mixture of oligosaccharide-alditols obtained by alkaline borohydride treatment of 510 mg of human meconium glycoproteins on a Bio-Gel P-6 column is shown in Fig. 1. The purification of the β-elimination products gives two fractions: 100 mg of FI and 87 mg of FII. The yield with respect to the starting material was 36%. The carbohydrate composition of these fractions was determined by GC (Table I). The fraction FI, corresponding to the high-molecular-mass oligosaccharides, is characterized by a small amount of N-acetylgalactosaminitol and the presence of mannose. The fraction FII possesses a molar carbohydrate composition in accordance with the structure of O-glycosidically linked oligosaccharides: a high content of N-acetylgalactosaminitol and a small amount of mannose residues.

HPLC on quaternary amine packings of the fraction FII obtained by Bio-Gel P-6 chromatography of the alkaline borohydride treatment of human meconium results in two major fractions, the neutral fraction FII-N and the acidic fraction FII-1 (Fig. 2). The results of the preparative chromatography of 48 mg of FII and the carbohydrate compositions of the fractions are given in Table II. The use of

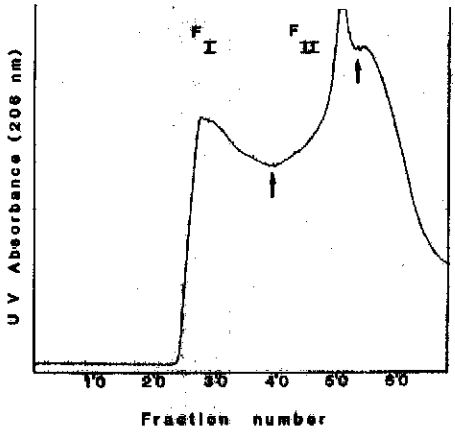

Fig. 1. Gel permeation of oligosaccharide-alditols obtained from human meconium glycoproteins by alkaline borohydride treatment on a Bio-Gel P-6.

TABLE I

CARBOHYDRATE COMPOSITIONS AND MASSES OF FRACTIONS OBTAINED BY BIO-GEL P-6 CHROMATOGRAPHY OF OLIGOSACCHARIDES RELEASED BY ALKALINE BOROHYDRIDE TREATMENT OF HUMAN MECONIUM

Fraction	Molar ratio*							Mass (mg)
	Fuc	Gal	Man	GalNAc	GlcNAc	NeuAc	GalNAc-ol	
FI	0.48	1	0.2	0.3	1.1	0.3	0.09	100
FII	0.48	1	0.12	0.15	1	0.23	0.46	87

*The molar ratio of Gal was taken as 1; Fuc = fucose; GlcNAc = N-acetylglucosamine; NeuAc = N-acetylneuraminic acid; GalNAc-ol = N-acetylgalactosaminitol.

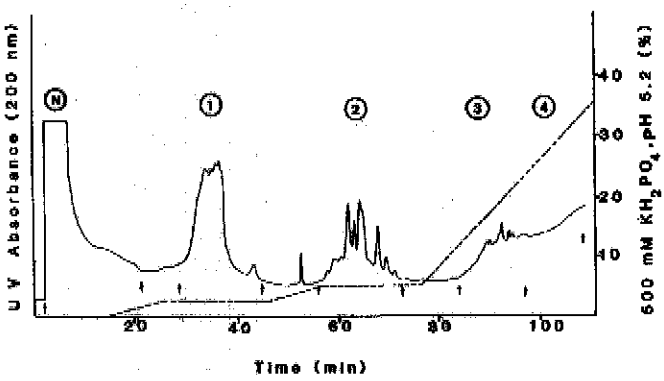

Fig. 2. Analysis of oligosaccharide-alditols obtained from β-elimination of human meconium (Bio-Gel P-6, FII fraction) on a 10-μm AX-10 column.

TABLE II

CARBOHYDRATE COMPOSITIONS AND MASSES OF FRACTIONS OBTAINED BY AX-10 PREPARATIVE CHROMATOGRAPHY OF OLIGOSACCHARIDE-ALDITOLS RELEASED BY ALKALINE BOROHYDRIDE TREATMENT OF HUMAN MECONIUM (FII)

Fraction	Molar ratio*							Mass (mg)
	Fuc	Gal	Man	GalNAc	GlcNAc	NeuAc	GalNAc-ol	
FII	0.48	1	0.12	0.15	1	0.23	0.46	48
FII- N	0.61	1	0.13	0.16	0.71	–	0.44	11.5
FII-1	0.53	1	0.12	0.14	1.08	0.38	0.44	7.7
FII-2	0.47	1	0.17	0.09	0.89	0.58	0.39	2.5
FII-3	0.45	1	0.18	0.08	0.82	0.38	0.15	0.5
FII-4	0.44	1	0.37	0.18	0.90	0.15	0.03	0.27

*The molar ratio of Gal was taken as 1.

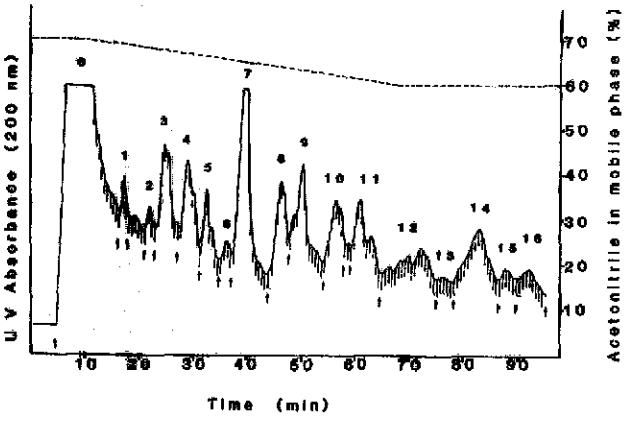

Fig. 3. Analysis of neutral oligosaccharide-alditols (FII-N) obtained from β-elimination of human meconium and anion-exchange chromatography on an Amino Zorbax column.

a column filled with silica modified by an organic quaternary amine provides a recovery of 47.5% for the oligosaccharide-alditols. The major fraction consists of neutral oligosaccharide-alditols (50%). Fractions FII-1 and FII-2 have the same chromatographic mobility as monosialylated oligosaccharides and disialylated oligosaccharides [9], and represent 34.3 and 11% of total oligosaccharide-alditols, respectively.

The neutral fraction (FII-N) from the AX-10 chromatography of oligosaccharide-alditols FII was subjected to HPLC using acetonitrile–water and alkylamine-modified silica. The effective separation of sixteen fractions was obtained in 80 min (Fig. 3). The carbohydrate composition and the mass of each fraction are given in Table III. The yield with respect to starting material (11.5 mg) was 48.6%.

In order to purify the oligosaccharide-alditol fractions obtained by HPLC on an alkylamine column, the fractions FII-N-3, -4, -5, -7, -8, -9, -10, -11 and -12 were subjected to HPLC on an octadecylsilyl column with water and water–acetonitrile as solvent. The results of the chromatographic separation of

TABLE III

CARBOHYDRATE COMPOSITIONS AND MASSES OF FRACTIONS OBTAINED BY SEMI-PREPARATIVE CHROMATOGRAPHY OF NEUTRAL OLIGOSACCHARIDE-ALDITOLS (FII-N) RELEASED BY ALKALINE BOROHYDRIDE TREATMENT OF HUMAN MECONIUM

Fraction	Molar ratio*						Mass (μg)
	Fuc	Gal	Man	GalNAc	GlcNAc	GalNAc-ol	
FII-N-0	2.38	5.2	0.7	2.68	5.6	1	84
-1	0.56	0.91	0.28	0.42	2.6	1	84
-2	1.12	1.49	0.3	0.28	1	1	62
-3	0.19	1.06	0.09	—	0.94	1	335
-4	0.65	1.08	0.1	—	1.0	1	300
-5	2	2	0.16	—	2.2	1	325
-6	1.67	2	0.2	0.24	2	1	58
-7	0.36	1.51	0.05	0.13	1.1	1	1000
-8	1.3	2	—	—	1.7	1	187
-9	1.37	2.3	—	—	1.8	1	170
-10	1.7	1.9	—	—	1.8	1	410
-11	1	2.6	—	0.64	2.3	1	160
-12	2	3.3	—	0.58	3.0	1	610
-13	1.8	2.8	0.35	0.51	2.3	1	185
-14	1.9	3.0	0.23	0.36	3.17	1	760
-15	1.5	2.3	0.8	0.3	1.95	1	240
-16	1.55	2.5	0.36	0.28	3.0	1	480
Tail	2	3.6	0.5	—	3.7	1	145
Recovery							5592

*The molar ratio of GalNAc-ol was taken as 1.

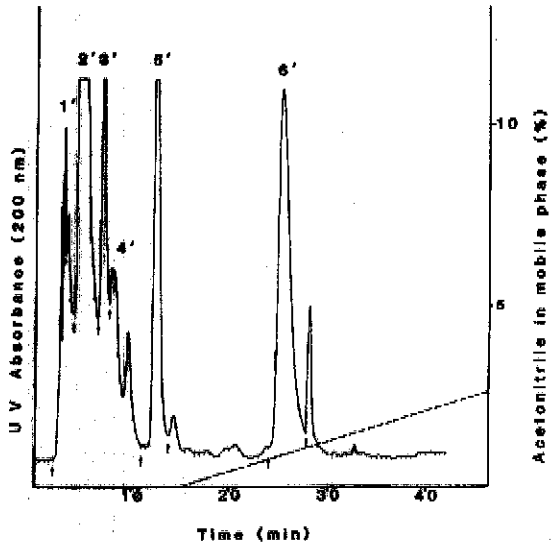

Fig. 4. Analysis of neutral oligosaccharide-alditols FII-N-7 obtained from β-elimination of human meconium, anion-exchange chromatography and normal-phase partition chromatography, by reversed-phase chromatography on a Zorbax ODS column.

TABLE IV

CARBOHYDRATE COMPOSITIONS OF FRACTIONS OBTAINED BY SEMI-PREPARATIVE CHROMATOGRAPHY ON OCTADECYLSILYL COLUMN OF NEUTRAL OLIGOSACCHARIDE-ALDITOLS FROM ALKYLAMINE-MODIFIED SILICA CHROMATOGRAPHY

Fraction	Molar ratio*					
	Fuc	Gal	Man	GalNAc	GlcNAc	GalNAc-ol
FII-N-3-1′	0.5	1	–	0.5	1	–
-2′	0.84	2.5	–	–	2.5	1
-3′	–	1	–	–	1	1
FII-N-4-2′	–	0.96	–	–	0.90	1
-3′	0.88	0.92	–	0.83	0.3	1
-4′	3.4	4.2	0.6	1.6	4.2	1
-5′	–	1	–	–	1.8	–
-6′	0.56	1	–	–	1.7	–
-7′	1.1	1.2	0.1	–	1	1
-8′	0.8	0.8	–	–	0.8	1
FII-N-5-2′	1	0.94	–	–	0.97	1
-3′	0.99	1.0	–	–	0.83	1
-4′	1	2.1	0.42	–	3.7	1
-5′	1.3	1.5	–	–	1.8	1
FII-N-7-1′	1	1.5	–	–	1	–
-2′	–	2	–	–	1	1
-3′	1.75	1.45	–	–	1.45	1
-4′	1.57	1.9	0.3	0.62	3.2	1
-5′	1.3	1	–	1.45	1.56	1
-6′	1	1.7	–	–	1	1
FII-N-8-2′	1.8	2	–	–	2.1	1
-3′	0.9	1.75	–	–	1.37	1
-4′	4	4.3	–	–	6.5	1
-5′	–	4.7	–	1	5.9	1
FII-N-9-1′	2.7	3.4	3	2.7	1.5	–
-2′	0.8	1.54	–	–	0.93	1
-3′	0.3	1.7	–	–	1.2	1
-4′	0.9	1.7	0.17	0.42	1.3	1
-5′	1.05	2.1	–	0.64	2.1	1
-6′	1.46	1.6	–	–	0.87	1
-7′	0.99	1.5	–	–	0.99	1
-8′	1.6	1.8	–	–	1.27	1
FII-N-10-1′	1.25	1.6	0.25	0.25	1	1
-2′	1.4	2	–	–	1.87	1
-3′	0.72	2.1	–	–	2.9	1
-4′	1.8	3.3	–	0.9	2.8	1
-5′	1	1.7	–	–	1.7	1
-6′	1.9	2.7	–	–	2	1
-7′	1.6	1.1	–	0.2	0.9	1
FII-N-11-3′	0.75	1	–	0.5	1	1
FII-N-12-2′	1.5	2.47	0.26	0.2	2.1	1
-3′	1.6	3.3	–	0.29	4	1
-4′	2.3	3.6	–	–	0.44	1
-5′	3.1	3	–	1.9	2.9	1
-6′	0.9	0.93	–	0.28	1.48	1
-7′	1.73	2.7	0.36	–	2.23	1

*The molar ratio of GalNAc-ol was taken as 1.

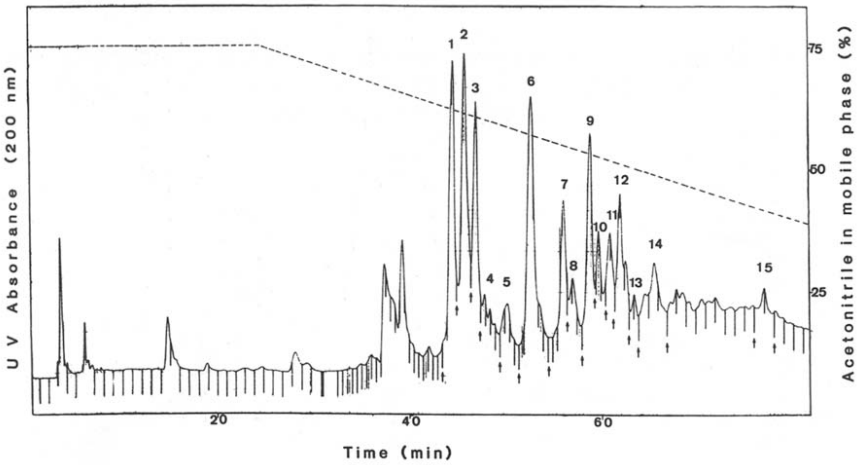

Fig. 5. Analysis of acidic oligosaccharide-alditols (FII-1) obtained from β-elimination of human meconium and anion-exchange chromatography on an Amino Zorbax column.

TABLE V

CARBOHYDRATE COMPOSITIONS AND MASSES OF FRACTIONS RELEASED BY SEMI-PREPARATIVE CHROMATOGRAPHY ON ALKYLAMINE-MODIFIED SILICA OF ACIDIC OLIGOSACCHARIDE-ALDITOLS (FII-1) OBTAINED BY ALKALINE BOROHYDRIDE TREATMENT OF HUMAN MECONIUM

Fraction	Molar ratio*							Mass (μg)
	Fuc	Gal	Man	GalNAc	GlcNAc	NeuAc	GalNAc-ol	
FII-1-1	–	–	–	1	–	1	1	300
-2	–	0.2	–	0.1	0.63	1	1	400
-3	–	0.76	–	–	0.23	1.08	1	290
-4	0.32	1	–	–	0.6	1.09	1	110
-5	0.49	1.3	–	0.39	1.23	0.73	1	120
-6	0.14	0.7	–	–	0.67	0.81	1	525
-7	–	1.88	–	–	1.0	1	1	180
-8	0.49	2	–	–	1.46	0.82	1	120
-9	0.83	1	–	–	1	1	1	330
-10	1	1.28	0.33	–	1	0.8	1	156
-11	0.8	2.4	0.3	0.15	1.2	1	1	240
-12	3.5	3.2	–	0.74	2.3	1.48	1	123
-13	2	2.84	–	0.7	2.7	1.49	1	100
-14	2.8	4.7	–	–	2.3	–	1	82
-15	2.34	4.85	1.8	–	3.8	1.2	1	110

*The molar ratio of GalNAc-ol was taken as 1.

the major fraction FII-N-7 are illustrated in Fig. 4, and Table IV gives the carbohydrate composition of each fraction. The use of a reversed-phase column provides good purification of each oligosaccharide-alditol fraction obtained by HPLC on an alkylamine column. For instance, the peak FII-N-7 (Fig. 3), which appears to be relatively pure from the alkylamine chromatography, gave six fractions (FII-

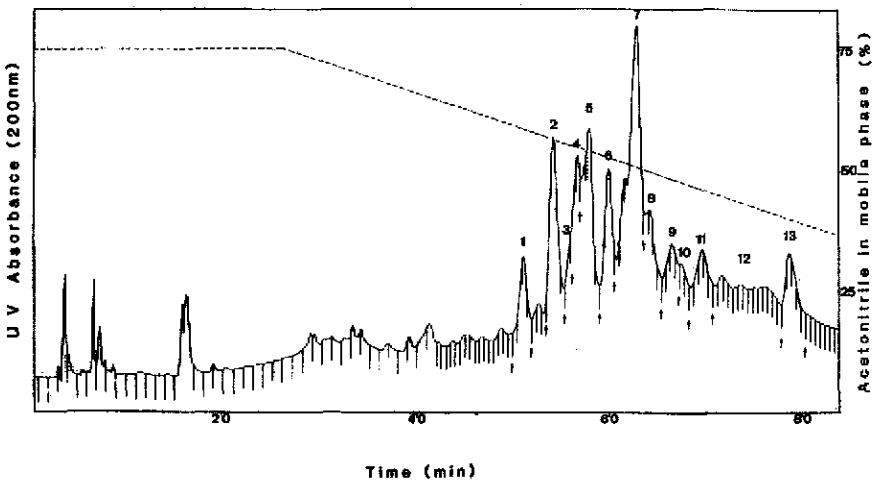

Fig. 6. Analysis of acidic oligosaccharide-alditols (FII-2) obtained from β-elimination of human meconium and anion-exchange chromatography on an Amino Zorbax column.

TABLE VI

CARBOHYDRATE COMPOSITIONS AND MASSES OF FRACTIONS RELEASED BY SEMI-PREPARATIVE CHROMATOGRAPHY ON ALKYLAMINE-MODIFIED SILICA OF ACIDIC OLIGOSACCHARIDE-ALDITOLS (FII-2) OBTAINED BY ALKALINE BOROHYDRIDE TREATMENT OF HUMAN MECONIUM

Fraction	Molar ratio*							Mass (μg)
	Fuc	Gal	Man	Glc	GlcNAc	NeuAc	GalNAc-ol	
FII-2-1	0.8	1.2	–	–	1	1.3	1	85
-2	0.2	1.2	–	–	–	2.2	1	110
-3	–	1.4	–	–	1.1	1.6	1	27
-4	–	1.2	–	–	0.95	2.1	1	90
-5	–	1.6	–	0.73	1	2	1	150
-6	–	1.6	–	0.8	1.2	2.2	1	145
-7	0.53	1	–	–	0.85	1.82	1	500
-8	0.58	1.7	–	1.4	1.2	1.7	1	155
-9	0.7	1.9	–	0.7	1.6	1.43	1	130
-10	0.8	2.1	–	–	2.1	1.3	1	50
-11	1.1	2.2	–	–	1.95	0.7	1	60
-12	1.5	2.7	0.47	–	2.8	1.1	1	175
-13	0.9	2.6	1.8	–	2.9	2	0.4	140

*The molar ratio of GalNAc-ol was taken as 1.

N-7-1′ to -6′, Fig. 4) on reversed-phase chromatography. Oligosaccharide-alditols FII-N-3-3′, FII-N-4-2′,8′, FII-N-5-2′,3′, FII-N-7-2′,6′, FII-N-8-2′, FII-N-10-3′ and FII-N-12-5′ were found to be homogeneous on the basis of HPLC and of the monosaccharide molar compositions.

In the neutral fraction, the oligosaccharide FII-N-3-3′ with galactose (Gal), N-acetylglucosamine (GlcNAc) and N-acetylgalactosaminitol (GalNAc-ol) in

a molar ratio of 1:1:1 and the oligosaccharide FII-N-7-2′ with Gal, GlcNAc and GalNAc-ol in a molar ratio of 2:1:1 possess the same carbohydrate composition of oligosaccharide fractions O4 and N1, respectively, isolated by Hounsell et al. [5] from meconium glycoproteins obtained from group O-secretors.

Other neutral oligosaccharides with the same core structure were isolated by Hounsell et al. [5] but with an additional monosaccharide. It is the case for the oligosaccharide FII-N-7-6′, which has the same carbohydrate composition as both oligosaccharide FII-N-7-2′ and N1 (Hounsell et al. [5]), plus an additional fucose residue.

The acidic fraction FII-1 from AX-10 chromatography of oligosaccharide-alditols FII was subjected to HPLC using acetonitrile–15 m*M* potassium dihydrogenphosphate as solvent and alkylamine-modified silica. The effective separation of fifteen fractions was obtained in 90 min (Fig. 5). The carbohydrate composition and mass of each fraction are given in Table V. Seven major fractions were obtained (FII-1-1, -2, -3, -6, -7, -9 and -11). The yield was 41.4%. Based on HPLC behaviour and carbohydrate compositions, fractions FII-1-1, -2, -3, -6, -7 and -9 appear to be in a pure state. These oligosaccharides also possess the same core composition as oligosaccharide fractions isolated from mild acid hydrolysis of meconium glycopeptides by Hounsell et al. [5] with additional sialic acid and fucose residues. The presence of Man and GalNAc-ol indicates that fraction FII-1-15 is a mixture of N- and O-glycosidically linked oligosaccharides.

The acidic fraction FII-2 from AX-10 chromatography was analysed by HPLC on an alkylamine-modified silica, under the same conditions as for the acidic fraction FII-1. Thirteen fractions were obtained (Fig. 6). The molar carbohydrate composition and the mass of each fraction are given in Table VI. The yield with respect to the starting material (2.5 mg) was 65.6%. This fraction FII-2 shows that these oligosaccharides possess the same chromatographic mobilities as disialyloligosaccharides.

However, several oligosaccharides do not contain 2 mol of sialic acid; this is the case for oligosaccharides FII-2-1, -3, -8, -10, -11 and -12.

The presence of sulphate residues cannot be excluded. The carbohydrate composition of fraction FII-2-13 shows the presence of Man. Like fraction FII-1-15, this fraction is a mixture of N- and O-glycosidically linked oligosaccharides.

The combination of ion-exchange, normal-phase and reversed-phase chromatography allows one to prepare oligosaccharide-alditols from human meconium in good yields. These oligosaccharide-alditols are, at present, being studied for the primary structure determination. The antigenic activities of these oligosaccharides will also be studied. For instance, the oligosaccharide fractions FII-N-10-3′, FII-1-6 and FII-1-9 contain a carbohydrate composition consistent with the glycan moiety of the antigenic determinant X found in human ovarian cyst glycoproteins [14].

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