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Note

Determination of the structures of fucosyl-lactose and difucosyl-lactose from the milk of monotremes, using ^{13}C-n.m.r. spectroscopy

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The milk carbohydrate of monotremes (egg-laying mammals: platypus and echidna) differs from that of marsupials and of placental mammals, notably in its high content of fucose[1]. The principal neutral carbohydrates of echidna and platypus milk are, respectively, fucosyl-lactose and difucosyl-lactose[1,2]. These compounds are also minor components of human milk[2–6]. Human milk contains two isomers of fucosyl-lactose[7], in one of which (2′-fucosyl-lactose)[3] α-L-fucose is linked to position 2 of D-galactose; in the other (3-fucosyl-lactose)[4], it is attached to position 3 of D-glucose. We now show by ^{13}C-n.m.r. spectroscopy that the trisaccharide from echidna milk is 2′-fucosyl-lactose (**1**) and that the tetrasaccharide from platypus milk is 3,2′-difucosyl-lactose (**2**).

The ^{13}C-n.m.r. spectrum (accumulated at 50.3 MHz for 64 h) of a 0.4% solution of the trisaccharide **1** (6 mg) in ^2H_2O contained twelve, readily observable resonances of approximately equal intensity, corresponding to the twelve carbons of the non-reducing sugar residues, and eleven resonances of small intensity (the α-peaks are also smaller than the β-peaks), corresponding to the reducing-end residue. One (δ 72.1) of the larger of the latter resonances apparently contained two coincident α-resonances. The chemical shifts and assignments are given in Table I.

The assignment procedure for **1** (in which D-glucose, D-galactose, and L-fucose are present in equimolar amounts[1]) commences with the reducing residue[8]. The chemical shift data for the eleven signals of the reducing residue are compared with those for the monosaccharides D-glucose, D-galactose, and L-fucose. Because of their downfield positions, the resonances involved in the linkage of the reducing sugar residue are those at δ 76.7 and 76.6 (α and β, respectively, based on their relative intensities); the signal of the carbon involved in a linkage is shifted downfield compared with that of the unsubstituted monosaccharide, and the magnitude is the same for both the α and β forms[8]. Furthermore, of the chemical shifts of the C-2,3,4 signals for the monosaccharides, only those of C-4α and C-4β are similar;

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TABLE I

^{13}C-CHEMICAL SHIFTS[a] AND ASSIGNMENTS FOR TRISACCHARIDE **1** AND TETRASACCHARIDE **2**

1		*C''* *α*-L-*Fuc*-(*1*→*2*)-*β*-D-*Gal*-(*1*→*4*)[b] *C'*		**2**	
Chemical shift	*Assignment*	*Chemical shift*	*Assignment*	*Chemical shift*	*Assignment*
101.1	1′	101.0	1′	101.0	1′
100.2	1″	100.1	1″	100.2	1″
				99.3, 99.2	1‴
96.7	1*β*			96.8	1*β*
92.6	1*α*			92.9	1*α*
				78.0	3*β*
77.1	2′	77.0	2′	77.2	2′
76.7	4*α*				
76.6	4*β*			76.4	4*α*,4*β*
76.1	5*β*				
76.0	5′	76.0	5′	75.7	5′
				75.6	3*α*
75.1	3*β*				
74.8	2*β*				
74.4	3′	73.1	4″[c]	74.5	3′
				73.6	5*β*[e]
				73.5	2*β*[e]
				72.9	4‴
72.5	4″	72.4	3′[c]	72.5	4″
72.1	2*α*,3*α*				
				71.6	2*α*
71.2	5*α*				
70.4	3″	70.4	3″	70.6	3″
70.0	4′	69.8	2″[d]	70.1	3‴
				69.6	4′,5*α*
69.0	2″	68.9	4′[d]	69.1	2″
				68.9	2‴
67.7	5″	67.9	5″	67.7	5″+5‴
61.9	6′	61.8	6′	62.3	6′
61.0	6*β*				
60.9	6*α*			60.8	6*α*,6*β*
16.1	6″	16.0	6″	16.3	6″,6‴

[a]Solution in ^2H_2O (internal 1,4-dioxane, *δ* 67.4); C, C′, C″, and C‴ correspond to the sugar residues as shown in **1** and **2**. [b]The chemical shifts are obtained from disaccharide fragments of two hexasaccharides given in ref. 9. [c,d]Pairs of residues in which the assignment due to Shashkov *et al.*[9] is the reverse of that given in this paper. [e]The assignments of these resonances may be reversed.

hence, the resonances *δ* 76.7 and 76.6 are assigned thereto. Linkage at position 6 is excluded because the C-6 resonances at *δ* 60.9 and 61.0 occur in their normal positions. The comparison of resonance positions shows that neither fucose nor galactose can be at the reducing end because of the lack of agreement between the

chemical shifts, particularly of the three β-resonances at δ 76.1, 75.1, and 74.8. On the other hand, the data for glucose fit well and, as expected[8], the residues adjacent to the linkage carbon atom are also shifted slightly upfield (3α and 3β by 1.7 and 1.6 p.p.m., respectively; 5α and 5β by 1.1 and 0.7 p.p.m., respectively).

The non-reducing-end residue was identified by comparison of the chemical shifts of the twelve single-carbon resonances and those of α- and β-L-fucose, and α- and β-D-galactose. The chemical shift of the signal for C-1″ would be expected to be 6–10 p.p.m. downfield from its position in the monosaccharide, that of the signal for C-2″ to be slightly shifted, and all other C″ resonances to be virtually unchanged. These criteria are met very closely only by α-fucose, since $\Delta\delta$ is −7.1 p.p.m. for C-1″, low for C-2″, and zero for the other resonances.

The middle residue must therefore be D-galactose, and the β form is probable because the resonances at δ 76.0 and 74.4 are consistent with those of C-5′ and C-3′ of β-D-galactose but not with any resonances of α-D-galactose. The very small downfield shift of 3.8 p.p.m. for C-1′ of **1** indicates that position 2 of the D-galactose is involved in a linkage, which would tend to reduce the downfield shift of the C-1′ resonance from its normal value of ~6 p.p.m. The downfield shift of the C-2′ resonance due to its linkage to α-L-fucose is 4.2 p.p.m., which is normal[8] for a linkage at position 2. The other resonances fit very well, confirming that the central residue is β-D-galactose substituted by α-L-fucose at position 2. The assignments in Table I are generally consistent with those of Shashkov *et al.*[9], who studied the n.m.r. spectra of oligosaccharides having terminal α-L-fucose residues (1→2)-linked to β-D-galactose. However, there are two pairs of resonances for which our assignments are the reverse of those given by Shashkov *et al.*[9]. The structure **1** is therefore assigned to the trisaccharide.

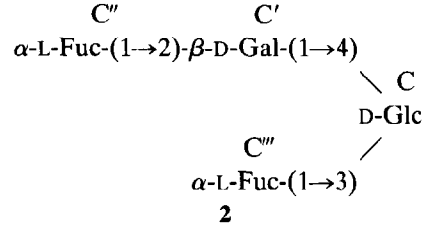
α-L-Fuc-(1→2)-β-D-Gal-(1→4)-D-Glc
C″ C′ C

1

The assignment of resonances in the ^{13}C-n.m.r. spectrum (accumulation time 64 h) of a 0.4% solution in ^2H_2O of the tetrasaccharide **2** (6 mg) (in which D-glucose, D-galactose, and L-fucose are present[1,2] in the molar ratios 1:1:2) is based on that of the trisaccharide (see Table I). Inspection of the large number of resonances which are common to **1** and **2** shows that the intensities of the C-5 and C-6 resonances at δ 67.7 and 16.3 due to L-fucose are doubled, and new resonances corresponding in position closely to those of the C-2,3,4 resonances of L-fucose in **1** also occur in **2**. This shows that the additional L-fucosyl group in **2** has an environment very similar to that in **1**, *i.e.*, it must be α and linked only at position 1‴. The appearance of one new, slightly split, C-1 resonance at δ 99.3, 99.2 is assigned to the additional L-fucosyl group, and the chemical shift confirms an α linkage. The splitting of this resonance is due to the two different configurations of the sugar residue to which it is linked. Since such splitting does not occur for **1**, in which D-galactose is linked to position 4 of the α and β forms of D-glucose (or in other previous exam-

ples of this type[10-12]), it is likely that the additional L-fucosyl group is linked[8] to the reducing D-glucose residue at either positions 2, 3, or 6. A 6-linkage is excluded because the 6α,6β resonances are in the same position as those for **1**. The splitting of the resonance at δ 99.3 is very small, which suggests[8,13] that the linkage is at position 3 rather than at position 2. This assessment is confirmed by the fact that the 1β and 1α resonances are virtually unchanged in **2** (see Table I), whereas, if the linkage were at position 2, a shift of these resonances would be expected. The resonances at δ 78.0 and 75.6 are assigned to C-3β and C-3α, which have undergone much smaller downfield shifts of 2.9 and 3.5 p.p.m., respectively, on linkage to fucose than the normal downfield shift of ~6 p.p.m. This is because of the linkage to galactose at position 4.

There is close correspondence between the chemical shifts of the resonances of **2** and **1** for the (1→2)-linked β-D-galactosyl residue (C′) and the C″ L-fucosyl group. Clearly, the additional L-fucosyl group (C‴) present in **2** is α- and linked to position 3 of the reducing D-glucose residue, which leaves the D-galactosyl residue (C′) and the C″ L-fucosyl group unchanged; hence **2** is the structure of the tetrasaccharide.



2

EXPERIMENTAL

Fucosyl-lactose was isolated by freeze-drying of the contents of peak 4 (Sephadex G-15) of Fig. 1a of ref. 1. Difucosyl-lactose was obtained by freeze-drying of the contents of peak 4 (Sephadex G-15) of Fig. 1b of ref. 1; further amounts of difucosyl-lactose were obtained by gel-permeation chromatography of platypus milk carbohydrates[2] on Bio-Gel P-4 (−400 mesh). The difucosyl-lactose had $[\alpha]_D^{18}$ −109° (*c* 1, water); *cf.* $[\alpha]_D^{20}$ −106° (water) for lactodifucotetraose[4].

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