Carbohydrate Research, 44 (1975) 101–105

Note

The 300-MHz n.m.r. spectra of melezitose and raffinose in deuterium oxide

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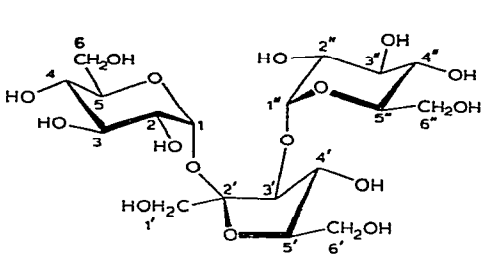
(Received February 24th, 1975; accepted for publication, June 10th, 1975)

^{1}H-N.m.r. spectral data for trisaccharides have been reported only for the Me_3Si derivatives of kestose and melezitose[1], and partially for acetylated kestose[2]. We now report on the 300-MHz spectra of melezitose and raffinose in D_2O. The spectral assignments were obtained by homo-INDOR experiments with refinement by SIMEQ 16/II simulations. The data are collected in Table I, and in Figs. 1 and 2.

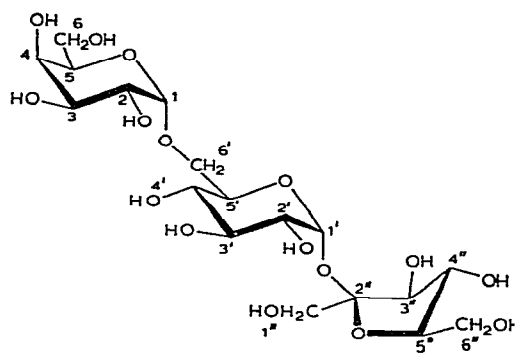
The signal of one of the glycosidic protons in both melezitose and raffinose is found at $\delta \sim 5.45$, a value also found for sucrose, and is assigned to H-1 in melezitose and H-1′ in raffinose. The second glycosidic proton is located at δ 5.18 in melezitose and δ 5.00 in raffinose. For turanose [α-D-glucopyranosyl-(1→3)-β-D-fructofuranose], a partial hydrolysis product of melezitose, although signals for glycosidic protons appeared at δ 5.22 and 5.31, depending on whether the α or β form is present, the signals could not be specifically assigned. However, it is likely that the signal at δ 5.22 represents the β isomer, since H-1″ in melezitose resonates at δ 5.18. The signals for the glycosidic protons in the anomers of melibiose in D_2O occur[3] at δ 4.98 and 4.99, *i.e.*, the same value as found for raffinose.

Melezitose (**1**). The observed shift values for H-2,2″,3,3″,4,4″,5,5″ in melezitose



1

Melezitose



2

Raffinose

coincide very well with those for the corresponding ring protons in turanose and sucrose[3], respectively. One exception is the signal for H-3 in sucrose, which occurs 0.2 p.p.m. to higher field (*cf.* +0.15 p.p.m. for trehalose).

The signals H-3′,4′ form the AB part of an ABX spin-system (X being H-5′), and the relevant coupling constants were obtained through computer-aided simulations, because classical calculation procedures failed. The values $J_{3',4'}$ 7.6 and $J_{4',5'}$ 8.0 Hz are typically high, indicating a ^4E(D) conformation for the fructofuranosyl moiety, corresponding to that[1] in the Me_3Si derivative of melezitose. The coupling constants for the pyranose ring protons are typical of the *C1*(D) conformation[4,5]. Although the AB-parts of the two ABX systems formed by H-5,5″ and H-6,6″ could be extracted from the spectrum, their similar chemical shifts preclude specific assignments. Nevertheless, the values $J_{A,X}\sim 2$ and $J_{B,X}\sim 5$ Hz are typical for α-D-glucopyranosyl fragments with HO-6 unsubstituted[3,4]. From the X pattern of the fructofuranosyl fragment, the sum (18 Hz) of the couplings for H-5′ is extractable

TABLE I

^{1}H-N.M.R. PARAMETERS OBTAINED AT 300 MHz FOR MELEZITOSE AND RAFFINOSE IN D_2O (TSP INTERNAL)

Chemical shifts	*H-1*	*H-2(H-1′)*	*H-3*	*H-4*	*H-5*	*H-6A*	*H-6B*
Melezitose							
Ring A (Glucopyranosyl)	5.45	3.56	3.67	3.44	3.92	3.90 3.86	3.78 3.79
Ring B (Fructofuranosyl)	3.81	3.65	4.32	4.30	3.92	3.85	3.85
Ring C (Glucopyranosyl)	5.18	3.58	3.75	3.45	3.92	3.86 3.90	3.79 3.78
Raffinose							
Ring A (Galactopyranosyl)	5.00	3.81	3.90	4.01	3.96	~3.75	~3.75
Ring B (Glucopyranosyl)	5.43	3.58	3.75	3.55	~4.07	~4.07	~3.71
Ring C (Fructofuranosyl)	3.68	3.68	4.23	4.03	3.90	3.85	3.78

Coupling constants	$J_{1A,1B}$	$J_{1,2}$	$J_{2,3}$	$J_{3,4}$	$J_{4,5}$	$J_{5,6A}$	$J_{5,6B}$	$J_{6A,6B}$
Melezitose								
Ring A	—[a]	3.8	10.0	8.8	10.2	2.2	4.8	~12.2
Ring B	—12.0	—[a]	—[a]	7.6	8.0	—[b]	—[b]	—[b]
Ring C	—[a]	3.8	10.0	9.0	10.0	2.2	4.8	~12.2
Raffinose								
Ring A	—[a]	3.8	10.0	3.0	1.0	—[b]	—[b]	—[b]
Ring B	—[a]	3.6	9.6	9.4	9.8	—[c]	—[c]	—[c]
Ring C	—[b]	—[a]	—[a]	8.4	8.0	2.4	7.6	—11.8

[a]Does not occur in this ring. [b]Not to be determined because of degeneracy. [c]Complex ABC system.

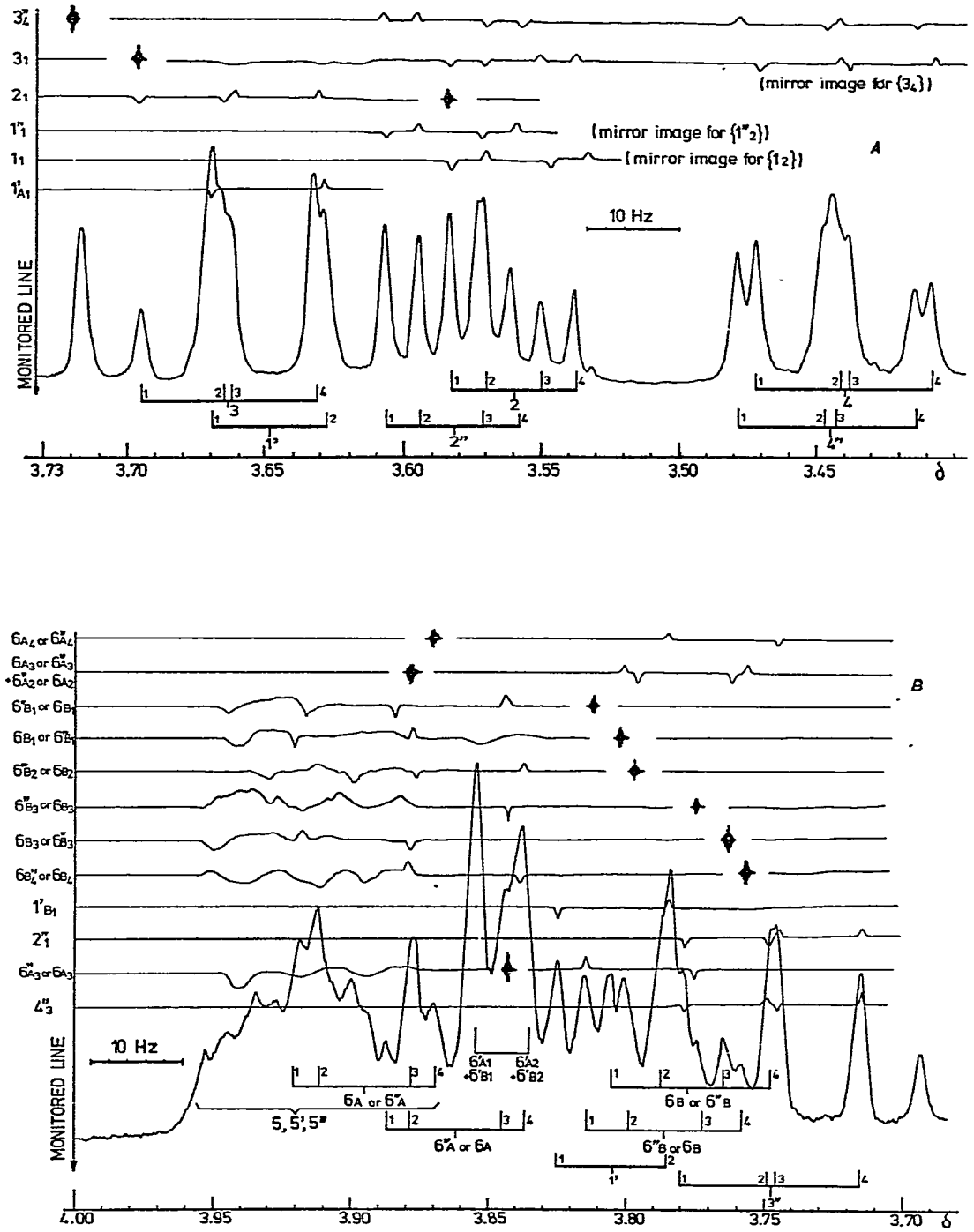


Fig. 1. Extended sweep of melezitose in D_2O at 300 MHz, and schematic representation of homo-INDOR experiments, with final assignments of protons. *A*, δ 3.40–3.70; *B*, δ 3.70–4.00.

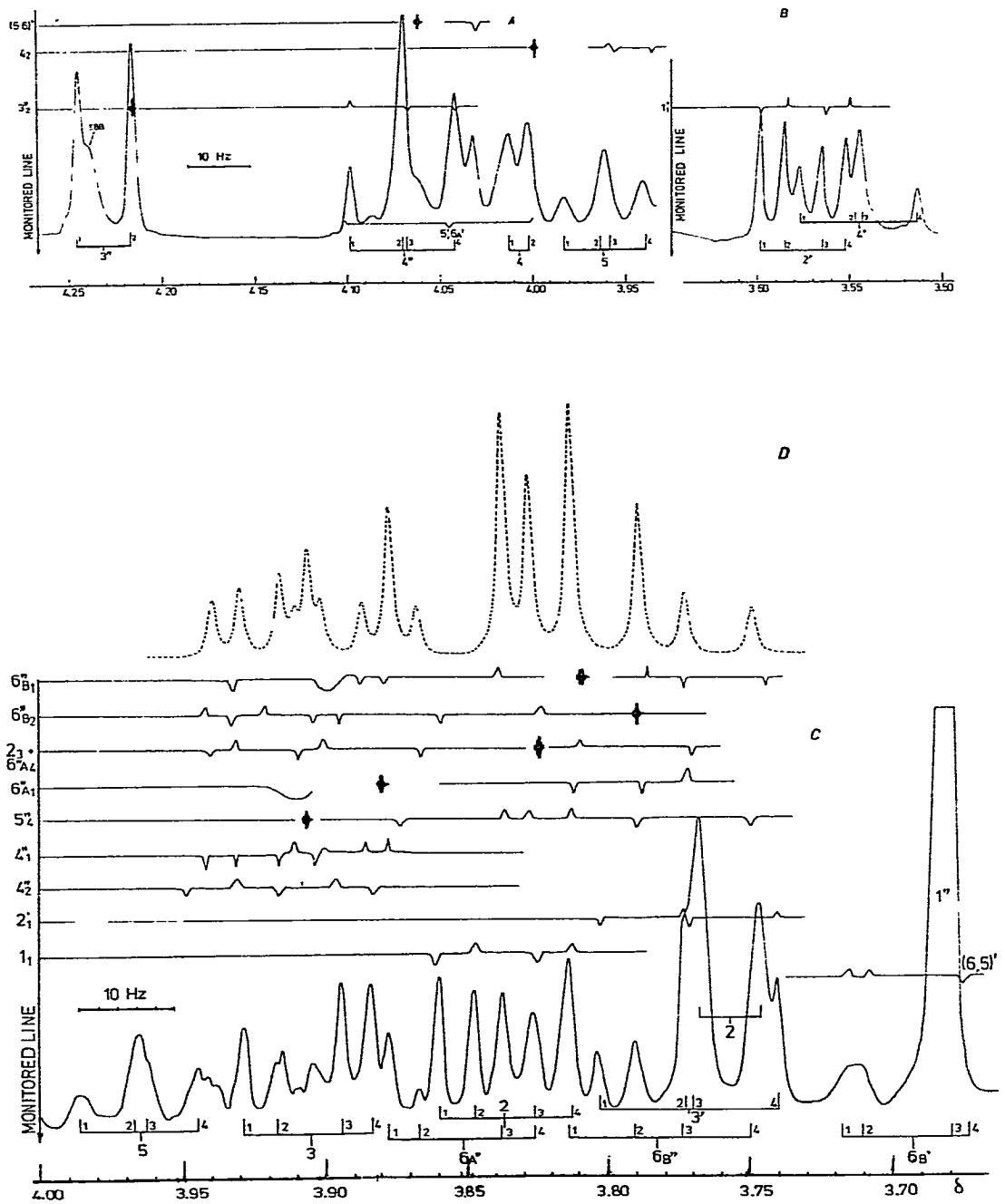


Fig. 2. Extended sweep of raffinose at 300 MHz, and schematic representation of homo-INDOR experiments, with final assignments of protons. *A*, δ 3.95–4.25; *B*, δ 3.50–3.65; *C*, δ 3.70–4.00; *D*, simulated spectral part (SIMEQ 16/II) for H-5″,6A″,6B″, using the values of Table I. The intensity of the simulated detail (*D*) is different from the original tracing (*C*). Line positions are explicitly indicated.

from homo-INDOR experiments, and since $J_{4',5'}$ is 8.0 Hz, it follows that $J_{A,X}+J_{B,X}=10.0$ Hz (the AB part is degenerate and appears only as two lines, separated by 5 Hz). This value is too large for a pyranosyl moiety, and thus the AB part belongs to the fructofuranosyl moiety. The value (0.16 p.p.m.) of $\Delta\delta$H-1′ is unusually large, the normal range being 0.01–0.03 p.p.m., except in turanose[3], where it is 0.11 p.p.m. in both anomers.

Raffinose (**2**). Irradiation (INDOR) of H-1 and H-1′ enabled identification of the signals for H-2,2′, and by repetition of this process the other pyranose ring protons were assigned, and compared with shift data obtained for α-D-gluco-, and α-D-galacto-pyranosides[4]. The pattern at δ 4.0–4.1 integrates for four protons (H-4,4″,5′,6′A; H-5′ being assessed by monitoring H-4′). By monitoring H-5′, responses were obtained within the same pattern (H-6′A), together with responses in the region δ 3.7. In view of the strongly coupled nature of the system, no further, more-precise data could be obtained. The δ values for H-5′ (~4.05) and H-6′B (~3.7) agree with the corresponding locations in melibiose (a partial hydrolysis product of raffinose); *cf.* isomaltose δ 3.97 (H-6A) and 3.77 (H-6B). The value of $J_{5',6'B}$ cannot be determined exactly, but it must be ~2 Hz, the same value as found for melibiose[3]. Monitoring the lines at δ ~3.9 gave responses (up or down) at slightly higher field (δ 3.8), each time with a spacing of 11.5–11.8 Hz, and therefore H-6″A,6″B are located at δ ~3.8. The calculated coupling values (subspectral analysis of H-5″, 6″A, 6″B as an ABM system) are $J_{5'',6''A}$~2.4 and $J_{5'',6''B}$~7.6 Hz. The sum of the outer lines of the pattern for H-5″ is again 18 Hz, *i.e.*, the sum of $J_{4'',5''}$, $J_{5'',6''A}$, and $J_{5'',6''B}$. These values are very close to those observed for the fructofuranosyl moiety of the TMS derivatives of fructose and melezitose[1]. Both protons on C-1″, which are located at δ 3.68, are isochronous, a situation paralleling that in sucrose.

From the values $J_{3'',4''}$ 8.4 and $J_{4'',5''}$ 8.0 Hz, it follows that the conformation of the fructofuranoside is ^4E(D). The remaining couplings for the pyranose rings indicate the expected $^4C_1$(D) conformation.

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