Carbohydrate Research, 97 (1981) 205–227
Elsevier Scientific Publishing Company, Amsterdam – Printed in The Netherlands

^{1}H- AND ^{13}C-N.M.R.-SPECTRAL STUDY OF SYNTHETIC METHYL D-MANNO-OLIGOSACCHARIDES*

TOMOYA OGAWA** AND KIKUO SASAJIMA***
The Institute of Physical and Chemical Research, Wako-shi, Saitama, 351 (Japan)
(Received May 19th, 1981; accepted for publication, June 16th, 1981)

ABSTRACT

^{1}H- and ^{13}C-n.m.r. spectra for 16 synthetic methyl manno-oligosaccharides were recorded, and the signals for the anomeric protons and anomeric carbon atoms in branched manno-pentaosides and -hexaosides were assigned, based on the data for methyl manno-biosides and -triosides. These n.m.r. data identified the branching pattern of high-mannose types of glycans of glycopeptides with those of unambiguously synthesized manno-oligosaccharides, and confirmed the structures proposed for such glycans.

INTRODUCTION

A variety of branched oligomannoside chains constitute part of the structure of such biologically important glycoconjugates as the high-mannose type of glycan of glycoproteins[2] and the cell-wall proteomannans of *Saccharomyces cerevisiae*[3] and *Piricularia oryzae*[4]. Recently, ^{1}H-n.m.r. spectroscopy has been found to be a powerful, analytical method for the elucidation of such glycan structures, mainly by Montreuil[5], Vliegenthart[6], Ballou[7], and their co-workers. In order to complement such studies, we now report ^{1}H- and ^{13}C-n.m.r. data obtained for 16 structurally well-defined, model compounds that had been synthesized by unambiguous routes in our laboratory, ranging from methyl mannobiosides to methyl mannohexaosides. Abbreviations used are given in Table I.

RESULTS AND DISCUSSION

A. ^{1}*H-N.m.r. spectra*

Four isomeric methyl α-D-mannopyranosyl-α-D-mannopyranosides, compounds **2** (ref. 8), **3** (ref. 9), **4** (ref. 10), and **5** (ref. 11), had been synthesized without ambiguity;

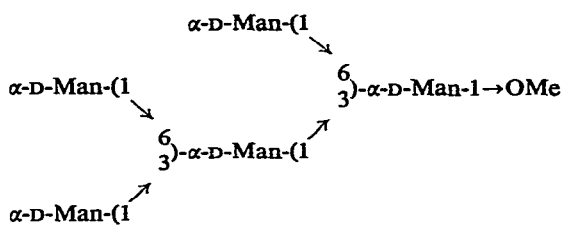
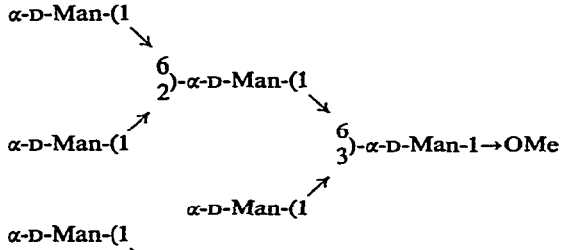
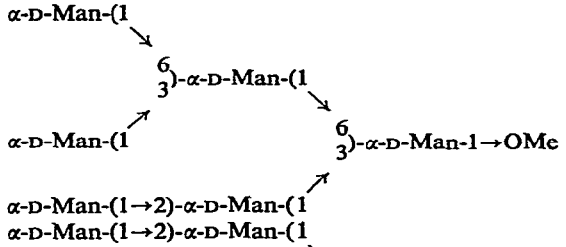
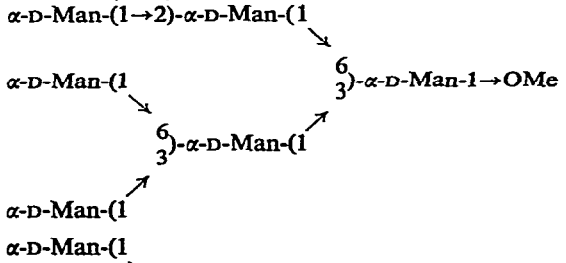
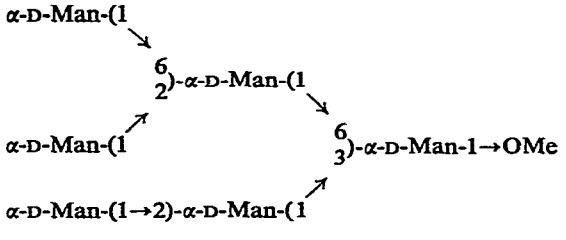
*Part 12 in the series "Synthetic Studies on Cell-surface Glycans". For Part 11, see ref. 1.
**To whom enquiries should be addressed.
***Present address: Sumitomo Chemical Co., Ltd., Fine Chemicals Div., Osaka Works, 3-1-98, Kasugade, Naka, Konohana-ku, Osaka, Japan.

TABLE I

STRUCTURES OF METHYL α-D-MANNO-OLIGOSACCHARIDES AND ABBREVIATIONS

Compound number	*Formula*	*Abbreviation*
2	α-D-Man-(1→2)-α-D-Man-1→OMe	b→2a
3	α-D-Man-(1→3)-α-D-Man-1→OMe	b→3a
4	α-D-Man-(1→4)-α-D-Man-1→OMe	b→4a
5	α-D-Man-(1→6)-α-D-Man-1→OMe	b→6a
6	α-D-Man-(1 ↘ 6 3)-α-D-Man-1→OMe ↗ α-D-Man-(1	b→6 c→3[a]
7	α-D-Man-(1 ↘ 6 2)-α-D-Man-1→OMe ↗ α-D-Man-(1	b→6 c→2[a]
8	α-D-Man-(1 ↘ 4 2)-α-D-Man-1→OMe ↗ α-D-Man-(1	b→4 c→2[a]
9	α-D-Man-(1→2)-α-D-Man-(1 ↘ 6 3)-α-D-Man-1→OMe ↗ α-D-Man-(1→2)-α-D-Man-(1	d→2b→6 e→2c→3[a]
10	α-D-Man-(1→2)-α-D-Man-(1 ↘ 4 2)-α-D-Man-1→OMe ↗ α-D-Man-(1→2)-α-D-Man-(1	d→2b→4 e→2c→2[a]
11	α-D-Man-(1→2)-α-D-Man-(1 ↘ 6 2)-α-D-Man-1→OMe ↗ α-D-Man-(1→2)-α-D-Man-(1	d→2b→6 e→2c→2[a]
12	α-D-Man-(1 ↘ 6 3)-α-D-Man-(1 ↘ α-D-Man-(1 6 3)-α-D-Man-1→OMe ↗ α-D-Man-(1	d→6 e→3b→6 c→3[a]

TABLE I *(continued)*

Compound number	*Formula*	*Abbreviation*
13		d→6[b→6, c→3a], e→3
14		d→6, e→2[b→6, c→3a]
15		d→6, e→3[b→6], f→2c→3a
16		d→2b→6, e→6[a], f→3c→3
17		d→6, e→2[b→6], f→2c→3a

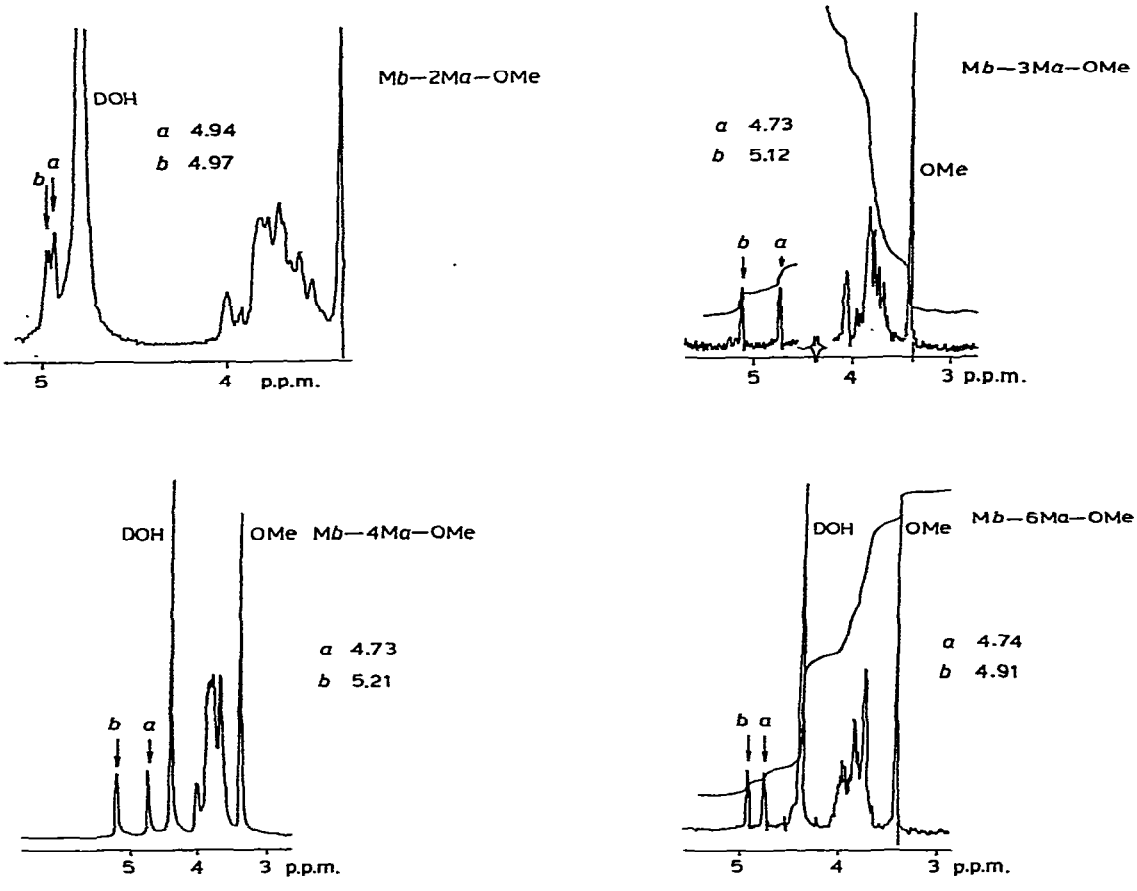


Fig.1. ^{1}H—N.m.r. spectra of mannobiosides (chemical shift values given in p.p.m.).

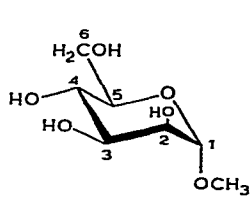
TABLE II

CHEMICAL SHIFTS OF ANOMERIC PROTONS OF METHYL MANNOBIOSIDES IN D_2O AT 60°

Proton	*Chemical shift (p.p.m.) for each structure*				
	b→2a (2)	b→3a (3)	b→4a (4)	b→6a (5)	1
H-1a	4.94 (+0.21)[a]	4.73	4.73	4.74	4.73
H-1b	4.97 (+0.24)[b]	5.12 (+0.39)[b]	5.21 (+0.48)[b]	4.91 (+0.18)[b]	—
OCH_3	3.40	3.41	3.40	3.40	3.40

[a]The value in parentheses for H-1a corresponds to the β-effect of mannosylation. [b]Values in parentheses for H-1b correspond to the amount of glycosidation shift: $\Delta\delta = \delta$ (H-1b) $- \delta$ (H-1 of **1**).

the ^{1}H-n.m.r. spectrum of each is shown in Fig. 1, and the chemical shift for the signals of each anomeric proton is listed in Table II. In the spectrum of each of the biosides **3**, **4**, and **5**, a signal, observed at the same chemical shift (δ 4.73–4.74 p.p.m.) as for the signal of H-1 in methyl α-D-mannopyranoside (**1**), was assigned to H-1a of each bioside. The other signal for the anomeric proton in **3**, **4**, and **5** was observed at δ 5.12, 5.21, and 4.91 p.p.m., respectively, and was assigned to H-1b. In the case of bioside **2**, signals at δ 4.94 and 4.97 p.p.m. were assigned to H-1a and H-1b, respectively, by comparing the signals for the anomeric protons in compounds **9**, **10**, and **11** (see Fig. 3), wherein all six anomeric protons of nonreducing mannosyl residues linked to O-2 of the internal mannosyl residues, expected to be in a magnetic environment similar to that of H-1b of **2**, gave signals at δ 5.00–5.03 p.p.m. (very close to δ 4.97). Deshielding of H-1a in the spectrum of bioside **2** by 0.21 p.p.m., compared to H-1 in **1**, may be explained by the favored conformation **2a**, stabilized by the exo-anomeric effect[12], wherein the ring-oxygen atom of the second mannosyl residue is close to H-1a. It may be noted that the observed glycosidation shift[13] [$\Delta\delta$ = δ(H-1b) — δ(H-1 of **1**)] for H-1b (see Table I) seems to be diagnostic in determining the site of mannosylation.



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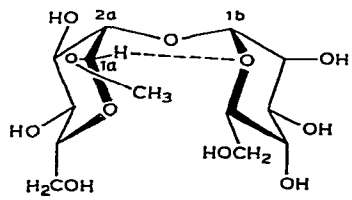


2a

TABLE III

CHEMICAL SHIFTS OF ANOMERIC PROTONS OF METHYL MANNOTRIOSIDES IN D_2O AT 60°

Proton	*Chemical shift (p.p.m.) for each structure*		
	b→6 c→3^a (6)	b→6 c→2^a (7)	b→4 c→2^a (8)
H-1a	4.70 (0)	4.96 (+0.23)[a]	4.95 (+0.22)
H-1b or c→2	—	5.04 (+0.31)[b]	5.01 (+0.28)
→3	5.11 (+0.38)	—	—
→4	—	—	5.22 (+0.49)
→6	4.91 (+0.18)	4.94 (+0.21)	—
OCH_3	3.40	3.40	3.39

[a]Values in parentheses for H-1a correspond to the β-effect. [b]Values in parentheses for H-1b and H-1c correspond to the glycosidation shift.

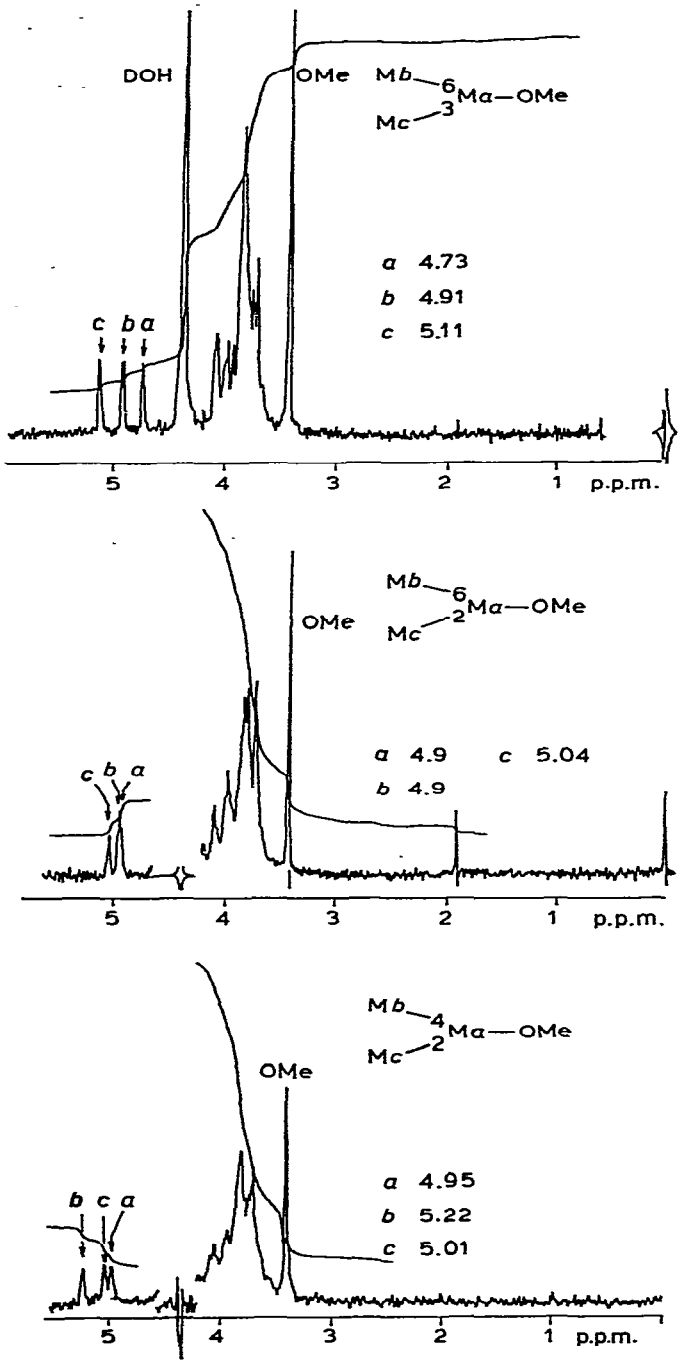


Fig. 2. ^{1}H-N.m.r. spectra of mannotriosides.

TABLE IV

GLYCOSIDATION SHIFTS (P.P.M.) OBSERVED FOR METHYL MANNOBIOSIDES AND METHYL MANNOTRIOSIDES

Proton	*Structure*		
	Triosides	*Biosides*	*Chemical shift (δ)*
H-1→2	+0.31 (7)[a], +0.28 (**8**)	+0.24 (**2**)	4.97–5.04
H-1→3	+0.38 (**6**)	+0.39 (**3**)	5.11–5.12
H-1→4	+0.49 (**8**)	+0.48 (**4**)	5.21–5.22
H-1→6	+0.18 (**6**), +0.21 (**7**)	+0.18 (**5**)	4.91–4.94

[a]Numbers in parentheses correspond to the compound that gave the data for the glycosidation shift.

^{1}H-N.m.r. spectra for methyl mannotriosides **6** (ref. 8), **7** (ref. 10), and **8** (ref. 10) are shown in Fig. 2, and the chemical shifts for anomeric protons are assigned in Table III from the data for the biosides, as follows. For trioside **6**, H-1a should remain at δ 4.73 p.p.m., as Man-a carries no mannosyl residue at O-2a. According to the glycosidation shift in Table II, H-1b and H-1c should appear at δ 4.91 and 5.12 p.p.m. Actually, two signals are present, at δ 4.91 and 5.11 p.p.m., in good agreement with expectation.

In the same way, from the data in Table II, the chemical shift expected for H-1a, H-1b, and H-1c for **10** and **11** will be δ 4.94, 4.91, and 4.97, and δ 4.94, 5.21, and 4.97 p.p.m., respectively. Actually, signals appear at δ 4.96, 4.94, and 5.04 p.p.m. for **10**, and δ 4.95, 5.22, and 5.01 p.p.m. for **11**, in moderate to good agreement with expectation.

For the biosides and triosides, the observed β-effect of mannosylation at O-2a on the chemical shift of H-1a was found to be in the range of +0.21 to +0.23 p.p.m. The glycosidation shifts observed for biosides and triosides are summarized in Table IV.

Six methyl mannopentaosides **9** (ref. 8), **10** (ref. 10), **11** (ref. 10), **12** (ref. 11), **13** (ref. 9), and **14** (ref. 14), and three methyl mannohexaosides **15** (ref. 11), **16** (ref. 9), and **17** (ref. 14) had been synthesized, and the ^{1}H-n.m.r. spectra of these oligosaccharides are shown in Figs. 3, 4, and 5. Signals for the anomeric protons were assigned by summing the glycosidation shift and β-effect of mannosylation observed for mannobiosides and -triosides. Thus, for pentaoside **9**, the signal for H-1a was expected to appear at δ 4.73 p.p.m., and the signals for H-1d and H-1e were expected to appear at δ 4.97–5.04 p.p.m. In actual fact, signals were observed at δ 4.70 p.p.m. for H-1a, and at δ 5.01 p.p.m. corresponding to two protons for H-1d and H-1e. The chemical shift for the signals of H-1b and H-1c on the internal mannosyl residues may be calculated by summing the glycosidation shift and β-effect due to 2-*O*-mannosylation. The expected chemical shifts are, then, (4.91–4.94) + (0.21 — 0.23) = 5.12–5.17 p.p.m. for H-1b and (5.11–5.12) + (0.21–0.23) = 5.32–5.35 p.p.m. for H-1c. Signals

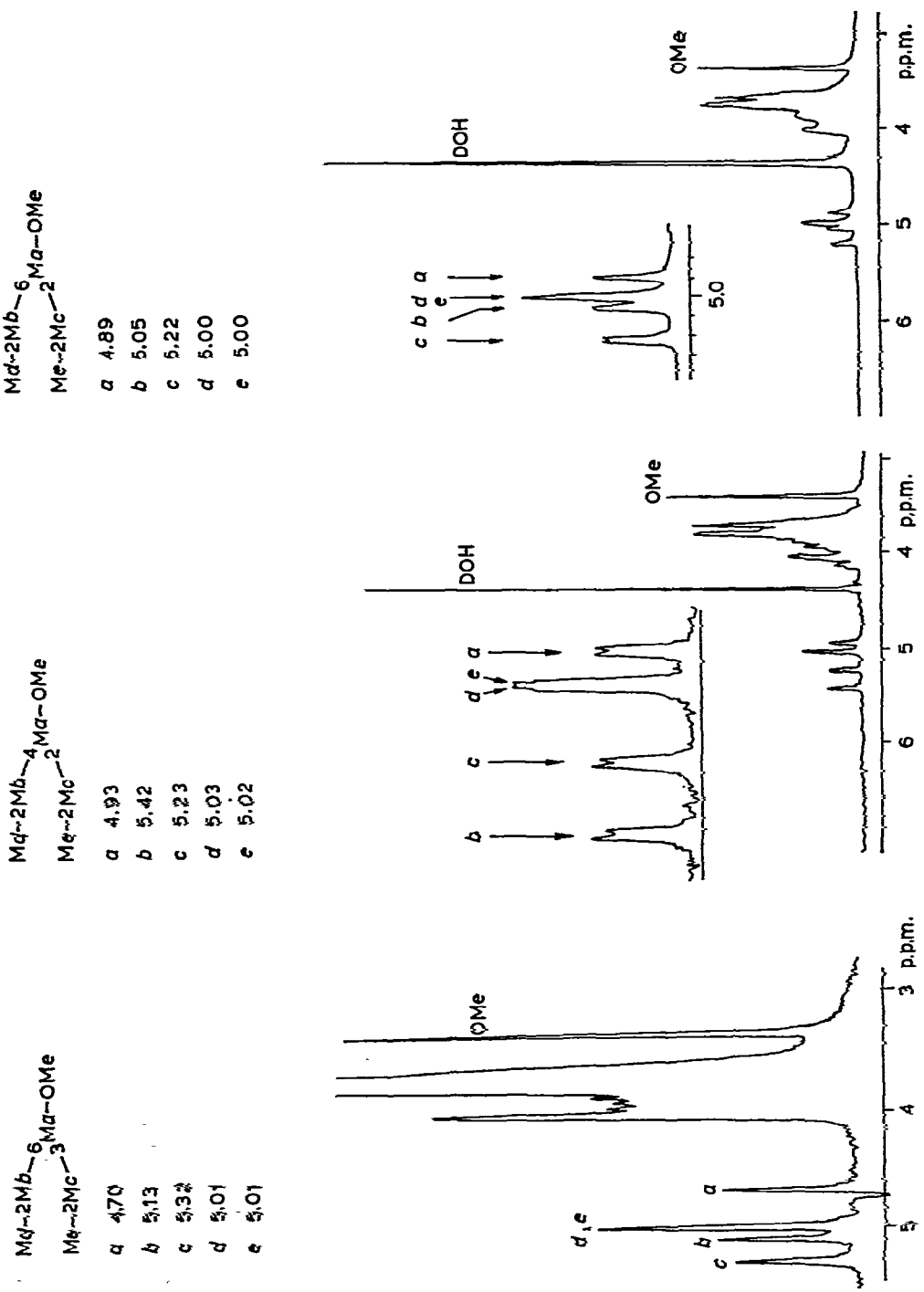


Fig. 3. ^{1}H-N.m.r. spectra of methyl mannopentaosides.

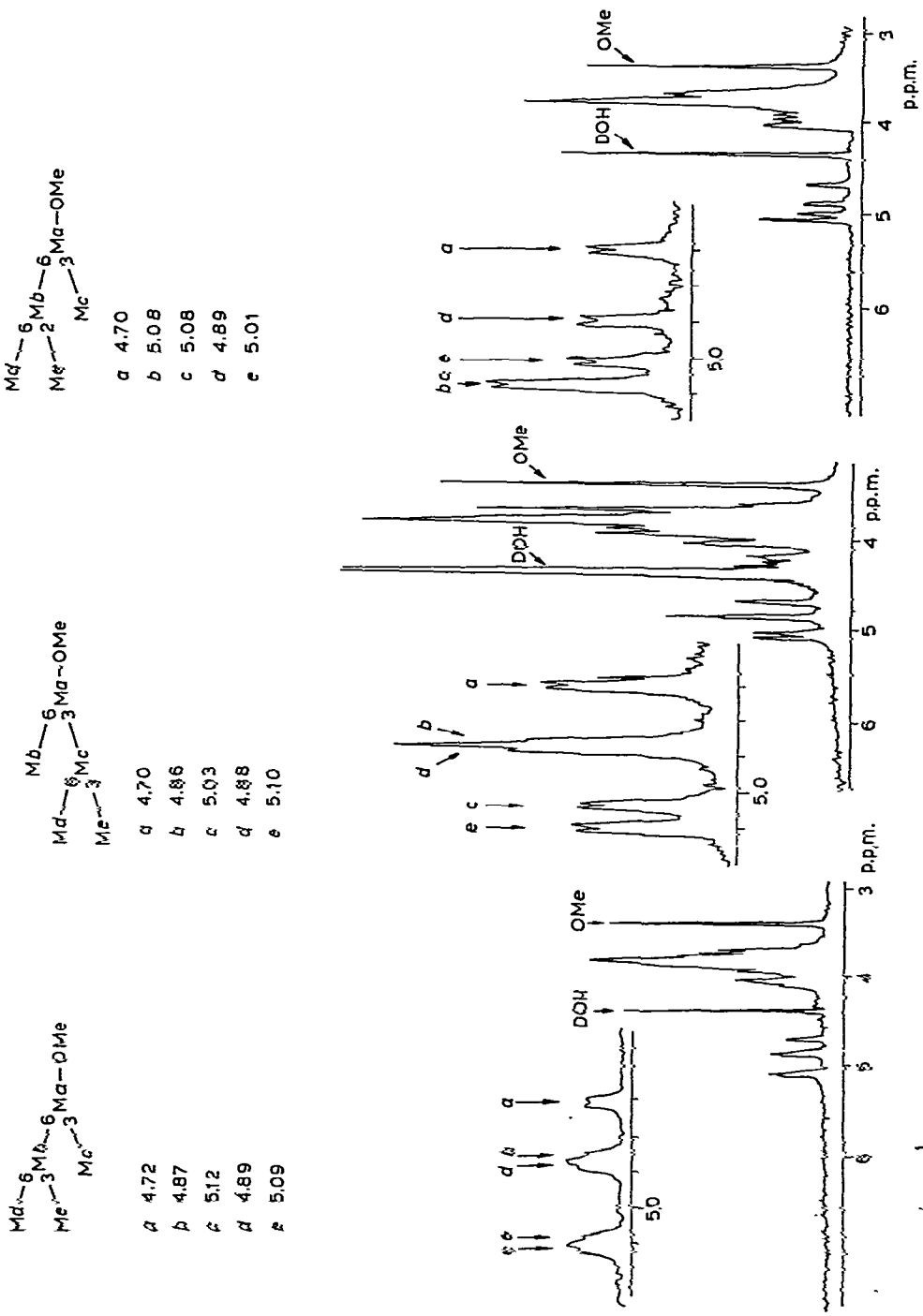


Fig. 4. ^{1}H—N.m.r. spectra of methyl mannopentaosides.

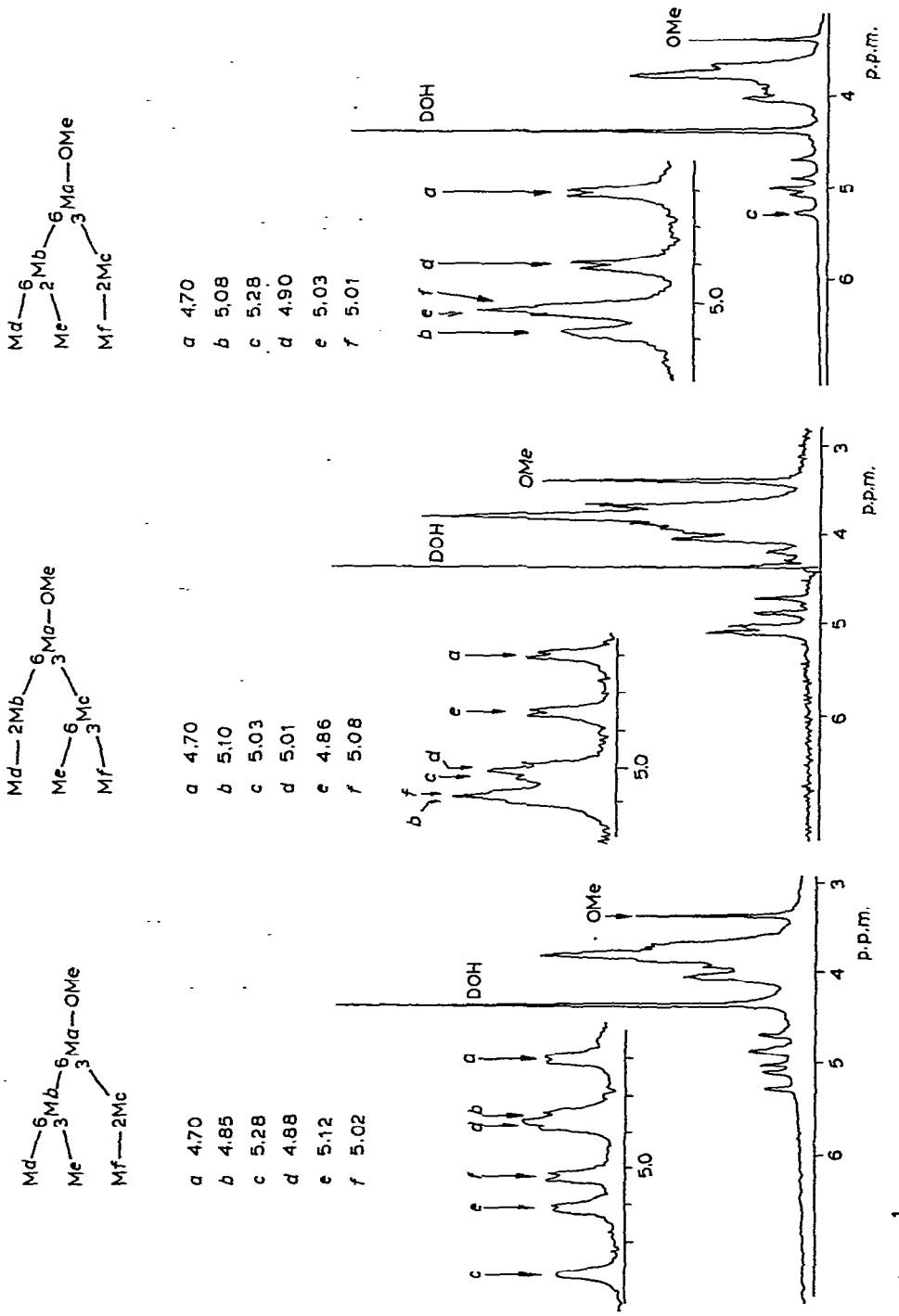


Fig. 5. ^{1}H–N.m.r. spectra of methyl mannohexaosides.

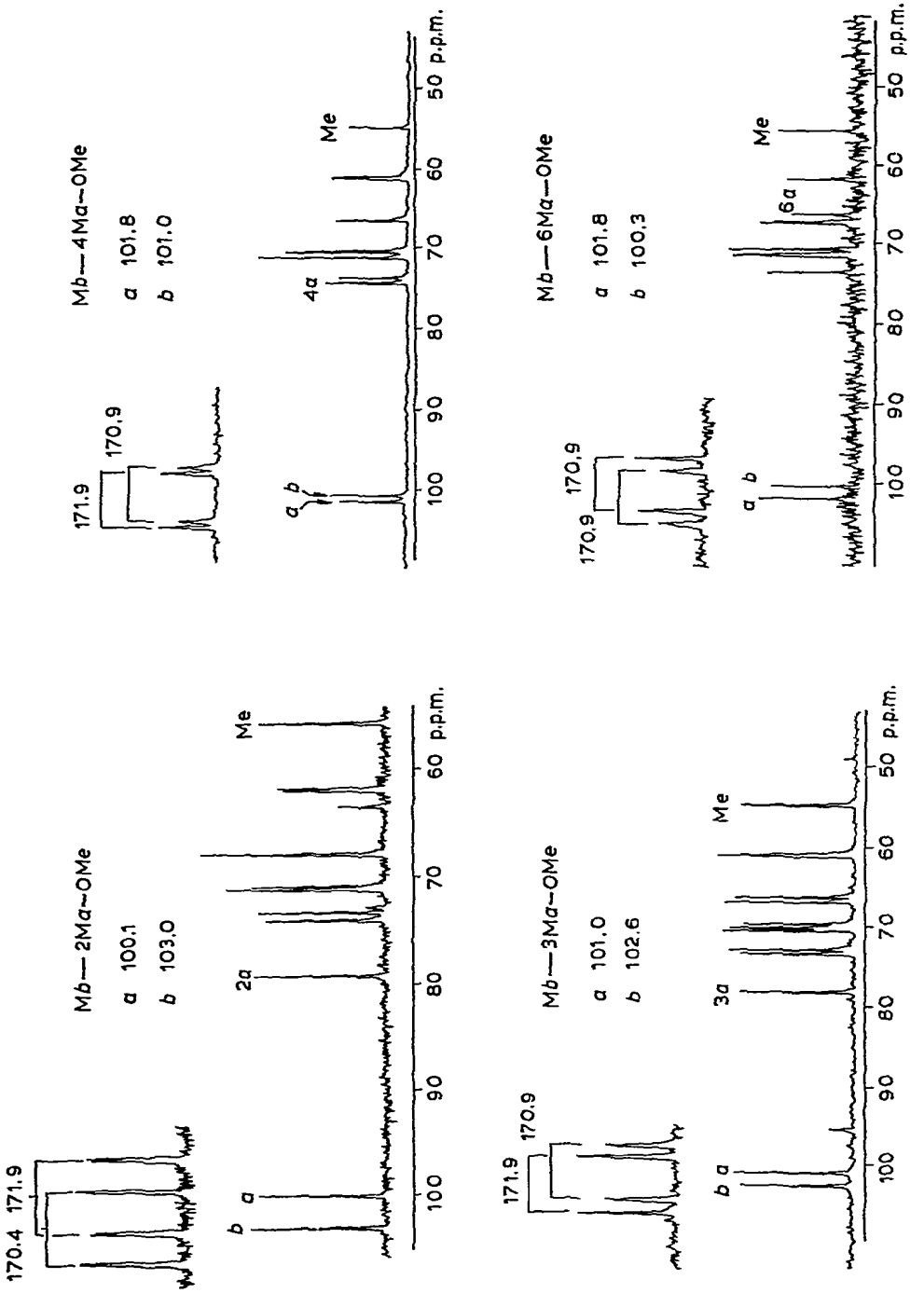


Fig. 6. ^{13}C—N.m.r. spectra of methyl mannobiosides.

TABLE V

OBSERVED AND CALCULATED (IN PARENTHESES) CHEMICAL SHIFTS FOR THE ANOMERIC PROTONS OF METHYL MANNOPENTAOSIDES AND METHYL MANNOHEXAOSIDES IN D_2O AT 60°

Compound	*Chemical shifts (p.p.m.) of each proton*						
	a	*b*	*c*	*d*	*e*	*f*	OCH_3
9	4.70 (4.73)	5.13 (5.12–5.17)	5.32 (5.32–5.35)	5.01 (4.97–5.04)	5.01 (4.97–5.04)		3.39
10	4.93 (4.94–4.96)	5.42 (5.42–5.45)	5.23 (5.18–5.27)	5.03*[a] (4.97–5.04)	5.02* (4.97–5.04)		3.39
11	4.89 (4.94–4.96)	5.05 (5.12–5.17)	5.22 (5.18–5.27)	5.00 (4.97–5.04)	5.00 (4.97–5.04)		3.37
12	4.72 (4.73)	4.87* (4.91–4.94)	5.12**[a] (5.11–5.12)	4.89* (4.91–4.94)	5.09** (5.11–5.12)		3.40
13	4.70 (4.73)	4.86* (4.91–4.94)	5.03** (5.11–5.12)	4.88* (4.91–4.94)	5.10** (5.11–5.12)		3.40
14	4.70 (4.73)	5.08 (5.12–5.17)	5.08 (5.11–5.12)	4.89 (4.91–4.94)	5.01 (4.97–5.04)		3.38
15	4.70 (4.73)	4.85* (4.91–4.94)	5.28 (5.32–5.35)	4.88* (4.91–4.94)	5.12 (5.11–5.12)	5.02 (4.97–5.04)	3.39
16	4.70 (4.73)	5.10 (5.12–5.17)	5.03** (5.11–5.12)	5.01 (4.97–5.04)	4.86 (4.91–4.94)	5.08* (5.11–5.12)	3.41
17	4.70 (4.73)	5.08 (5.12–5.17)	5.28 (5.32–5.35)	4.90 (4.91–4.94)	5.03* (4.97–5.04)	5.01* (4.97–5.04)	3.38

[a]Assignments described with * or ** may have to be interchanged.

for H-1b and H-1c were actually observed at δ 5.13 and 5.32 p.p.m., in good agreement with expectation.

In the same way, the chemical shifts expected and the data observed for the anomeric protons of methyl mannopentaosides and methyl mannohexaosides (see Table V) are in generally good agreement. The observation of the β-effect of mannosylation on the chemical shift of anomeric protons for the internal mannose residues of pentaosides and hexaosides strongly indicates that the orientation of interglycosidic linkages in manno-oligosaccharides is defined by the exo-anomeric effect, as in the case of bioside **2** (see **2a**). A deviation of over 0.05 p.p.m. between the expected and the observed chemical shifts is found for the signals at δ 5.05 p.p.m. (H-1b) for **11**, 5.03 p.p.m. (H-1e) for **13**, 4.85 p.p.m. (H-1b) for **15**, and 5.03 p.p.m. (H-1c) for **16**. These discrepancies may be ascribed to the specific conformations of the glycan chains.

In conclusion, the ^{1}H-n.m.r. signals of nine synthetic methyl manno-oligosaccharides having branched structures were reasonably assigned from the data for methyl manno-biosides and -triosides. These assignments were found to be in agreement with the signal assignments for the high-mannose type of glycans of glycopeptides recently reported by Montreuil[5], Vliegenthart[6], Ballou[7], and their co-workers, thus providing solid support, based on chemical synthesis, for their proposed structures.

TABLE VI

^{13}C-CHEMICAL SHIFTS FOR METHYL MANNOBIOSIDES IN D_2O AT 25°

Carbon atom	*Chemical shifts (p.p.m.) for each structure*									
	b-2a		*b-3a*		*b-4a*		*b-6a*		**1**	
1a	100.1	(−1.8)[a]	101.0	(−0.9)	101.8	(−0.1)	101.8	(−0.1)	101.9	
$^1J_{CH}$ (Hz)	171.9		170.9		171.9		170.9			
2a	79.3	(+8.1)	69.8	(−1.4)	71.4	(+0.2)	70.8	(−0.4)	71.2	
3a	70.8	(−1.0)	78.5	(+6.7)	70.7	(−1.1)	71.5*[b]	(−0.3)	71.8	
4a	67.8	(−0.2)	66.4*	(−1.6)	74.5	(+6.5)	67.4**[b]	(−0.6)	68.0	
5a	73.4*	(−0.3)	73.0**	(−0.7)	71.4	(−1.3)	71.6*	(−2.1)	73.7	
6a	61.9**	(−0.2)	61.1	(−1.0)	61.3	(−0.8)	66.5	(+4.4)	62.1	
1b	103.0	(+1.1)	102.6	(+0.7)	101.0	(−0.9)	100.3	(−1.6)		
$^1J_{CH}$ (Hz)	170.4		171.9		170.9		170.3			
2b	71.7	(+0.5)	70.3	(−0.9)	70.7	(−0.5)	70.8	(−0.4)		
3b	71.7	(−0.1)	70.6	(−1.2)	71.4	(−0.4)	71.5*	(−0.3)		
4b	67.8	(−0.2)	67.0*	(−1.0)	70.7	(+2.7)	67.7**	(−0.3)		
5b	74.1*	(+0.4)	73.6**	(−0.1)	74.0	(+0.3)	73.6	(−0.1)		
6b	61.8**	(−0.3)	61.1	(−1.0)	61.3	(−0.8)	61.8	(−0.3)		
OMe	55.7		55.0		55.0		55.7			

[a]Values in parentheses correspond to $\Delta\delta = \delta$(Cn) − δ(Cn of **1**). [b]Assignments marked with * or ** may have to be interchanged.

B. *^{13}C-N.m.r. spectra*

In 1973, Gorin[15] reported the rationalization of the ^{13}C-n.m.r. data for non-branched manno-oligosaccharides derived from yeast mannans by partial acetolysis. We now report the ^{13}C-n.m.r. data obtained for sixteen synthetic methyl manno-oligosaccharides. The ^{13}C-n.m.r. spectra of four methyl mannobiosides (**2**, **3**, **4**, and **5**) are shown in Fig. 6. The assignment of each signal was based on the data published[16] for methyl α-D-mannopyranoside, shown in Table VI, from which the following important observations were made. First, $^{1}J_{CH}$ values for all anomeric carbon atoms of α-D-mannopyranosyl residues were found to be between 170.4 and 171.9 Hz, in agreement with an observation of Bock and co-workers[17]. Second, the chemical shift of the carbinol carbon atom of a methyl α-D-mannopyranoside residue, to which another α-D-mannopyranosyl residue is linked, was found to be significantly deshielded (α-effect), and these signals could be readily differentiated from other signals of ring-carbon atoms. These data demonstrated that the substitution pattern of such compounds as biosides **2**, **3**, **4**, and **5** could be assigned from the magnitude of the α-effect. Third, signals for C-1a, on mannosylation at O-2a or O-3a, were shielded (β-, or γ-effect) by 1.8 and 0.9 p.p.m., whereas on mannosylation at O-4a or O-6a, they are uninfluenced. Fourth, signals for C-1b were shifted depending on the site of mannosylation (glycosidation shift), and those linked to O-2a and O-3a were deshielded by 1.1 and 0.7 p.p.m., whereas those linked to O-4a and O-6a were shielded by 0.9 and 1.6 p.p.m., respectively.

TABLE VII

^{13}C-CHEMICAL SHIFTS FOR METHYL MANNOTRIOSIDES IN D_2O AT 25°

Carbon atom	*Chemical shifts (p.p.m.) for each structure*		
	b-6 c-3^{a} (6)	b-6 c-2^{a} (7)	b-4 c-2^{a} (8)
1a	101.8 (−0.1)[a]	99.8 (−2.1)	99.4 (−2.5)
$^{1}J_{CH}$ (Hz)	172.9	171.9	172.0
2a	—	79.0 (+7.8)	79.4 (+8.2)
3a	79.4 (+7.6)	—	—
4a	—	—	74.9 (+6.9)
6a	64.9 (+2.8)	65.4 (+3.3)	—
1b,c→2	—	102.6 (+0.7)	102.6 (+0.7)
$^{1}J_{CH}$ (Hz)	—	169.9	169.9
1b,c→3	103.2 (+1.3)	—	—
$^{1}J_{CH}$ (Hz)	171.9	—	—
1b,c→4	—	—	101.8 (−0.1)
$^{1}J_{CH}$ (Hz)	—	—	170.9
1b,c→6	100.2 (−1.7)	99.8 (−2.1)	—
$^{1}J_{CH}$ (Hz)	169.9	171.9	—
OMe	55.7	55.2	55.2

[a]Values in parentheses correspond to $\Delta\delta = \delta(Cn) - (Cn \text{ of } \mathbf{1})$.

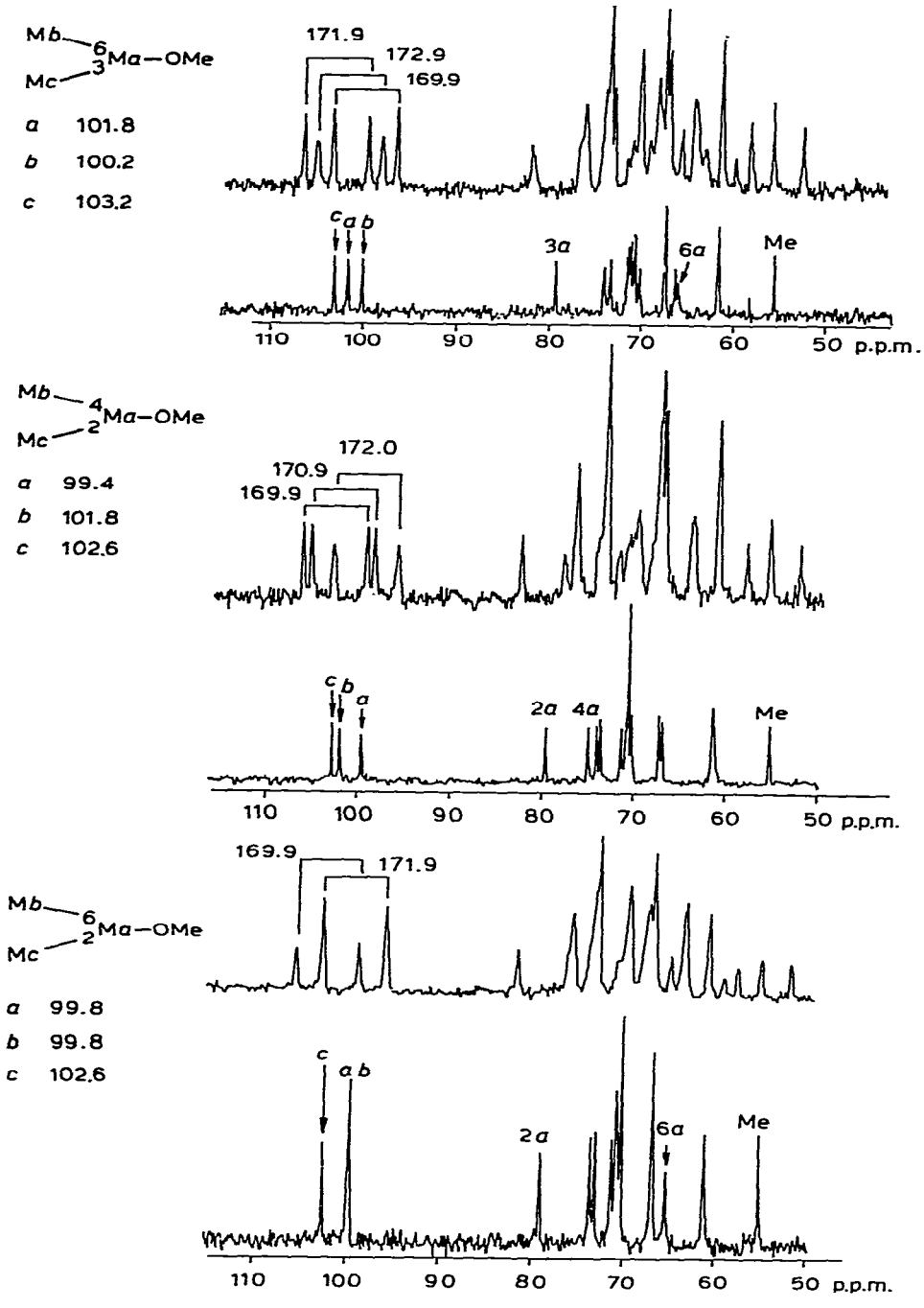


Fig. 7. ^{13}C—N.m.r. spectra of methyl mannotriosides.

The ^{13}C-n.m.r. spectra of methyl mannotriosides **6**, **7**, and **8** are shown in Fig. 7. Signal assignments, based on the data given in Table VI are shown in Table VII. Thus, for trioside **6**, C-1a should be shielded by 0.9 p.p.m. due to the γ-effect of mannosylation at O-3a, and should appear at $\delta \sim 101.0$ p.p.m. The signal for C-1b may be similar to that of **5**, and should appear at $\delta \sim 100.3$ p.p.m. The signal for C-1c should appear at $\delta \sim 102.6$ p.p.m., as in the case of **3**. Three signals were actually observed, at δ 100.2, 101.8, and 103.2 p.p.m., which were therefore assigned to C-1b, C-1a, and C-1c, respectively.

For trioside **7**, the signal for C-1a would be expected to be shielded, due to the β-effect of 2-*O*-mannosylation, and to appear at $\delta \sim 100.1$ p.p.m. along with C-1b at δ 100.3, and C-1c at δ 103.0 p.p.m., as with biosides **5** and **2**, respectively. In actual fact, two signals were observed, at δ 99.8 p.p.m. for two carbon atoms, and at δ 102.6 p.p.m. for one carbon atom, which were therefore assigned to C-1a and C-1b, and C-1c, respectively.

Similarly, for the trioside **8**, signals for C-1a, C-1b, and C-1c would be expected to appear at δ 100.1, 101.0, and 103.0 p.p.m., respectively, according to the data for methyl mannobiosides. In fact, three signals were observed, at δ 99.4, 101.8, and 102.6 p.p.m. As the signals at δ 99.4 and 102.6 p.p.m. could be assigned to C-1a and C-1c, that at δ 101.8 p.p.m. was assigned to C-1b.

From the ^{13}C-n.m.r. data for methyl mannobiosides and methyl mannotriosides, shown in Tables VI and VII, the following empirical rules may be advanced.

(*i*) *α-Effect of mannosylation.* — On α-D-mannopyranosylation, signals for C-2, C-3, C-4, and C-6 of the α-D-mannopyranosyl residue are deshielded by 7.7–8.8, 6.7–7.5, 6.5–7.2, and 3.3–4.4 p.p.m., and appear at δ 78.9–80.0, 78.5–79.3, 74.5–75.2, and 65.4–66.5 p.p.m., respectively.

(*ii*) *β- and γ-Effect of mannosylation.* — On α-D-mannopyranosylation at O-2a and O-3a, signals for C-1a are shielded by 1.7–2.2 and 0–0.6 p.p.m. (β- and γ-effect), whereas no shielding of the signal of C-1a is observed on α-D-mannopyranosylation at O-4a or O-6a.

(*iii*) *Glycosidation shift.* — The chemical shifts for the signals of C-1 are dependent on the site of glycosidic linkage. When compared with the signal for the anomeric carbon atom of methyl α-D-mannopyranoside, signals for anomeric carbon atoms attached at O-2 and O-3 of α-D-mannopyranosyl residues are deshielded by 0.1–1.1 and 0.6–1.4 p.p.m., and appear at δ 102.5–103.0 and 102.5–103.3 p.p.m., respectively. On the other hand, the signals for anomeric carbon atoms linked to O-4 and O-6 are shielded by 0.1–0.9 and 1.7–2.3 p.p.m., and appear at δ 101.0–101.8 and 99.6–100.2 p.p.m., respectively.

The ^{13}C-n.m.r. spectra of methyl mannopentaosides **9**–**14** and methyl mannohexaosides **15**–**17** are shown in Figs. 8, 9, and 10. The assignments for the anomeric carbon atoms of these methyl manno-oligosaccharides are based on the aforementioned, empirical rules *ii* and *iii*.

The chemical shift expected for C-1 of internal α-D-mannopyranosyl residues situated in the α-mannan chains may be calculated by summing the glycosidation

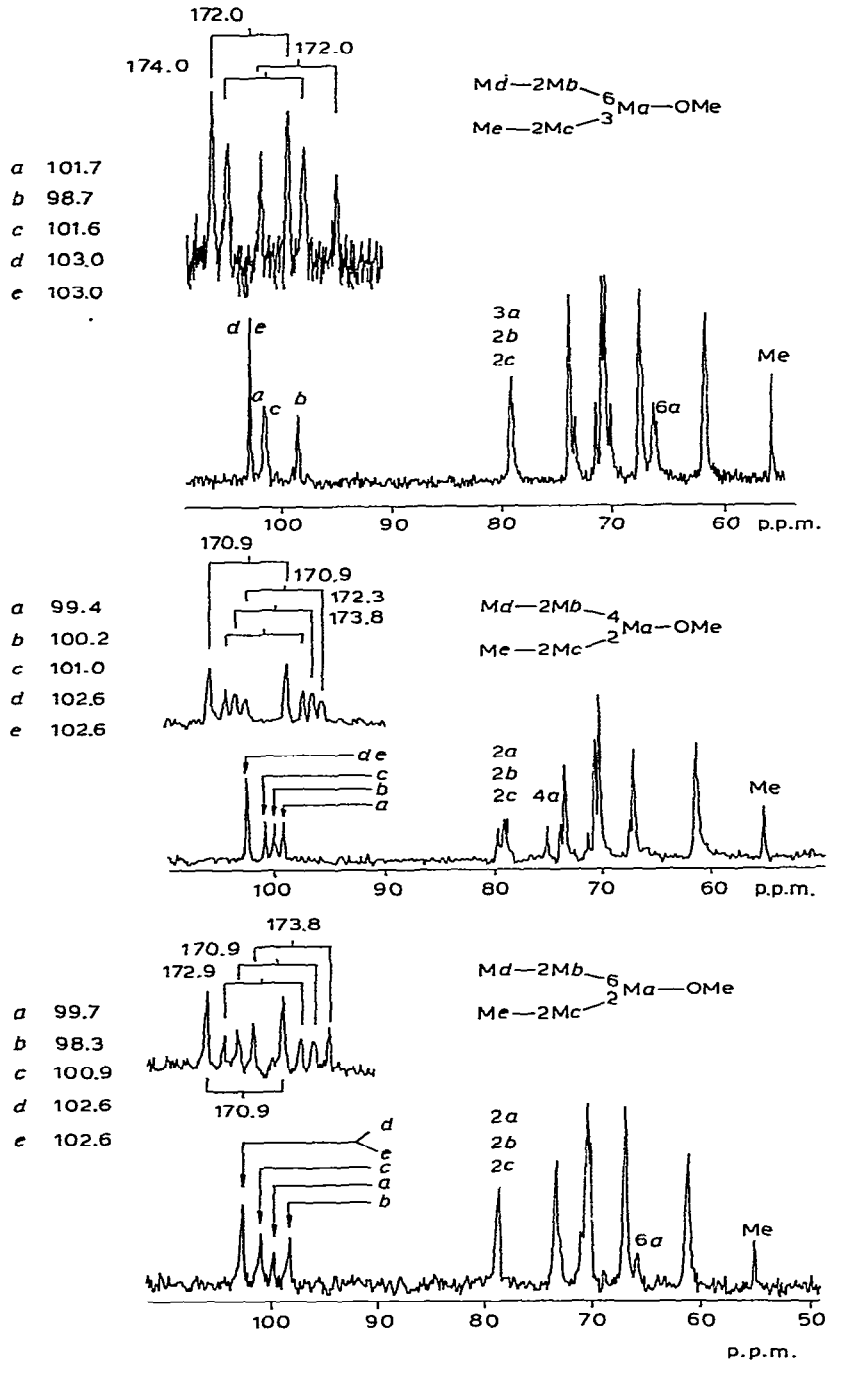


Fig. 8. ^{13}C–N.m.r. spectra of methyl mannopentaosides.

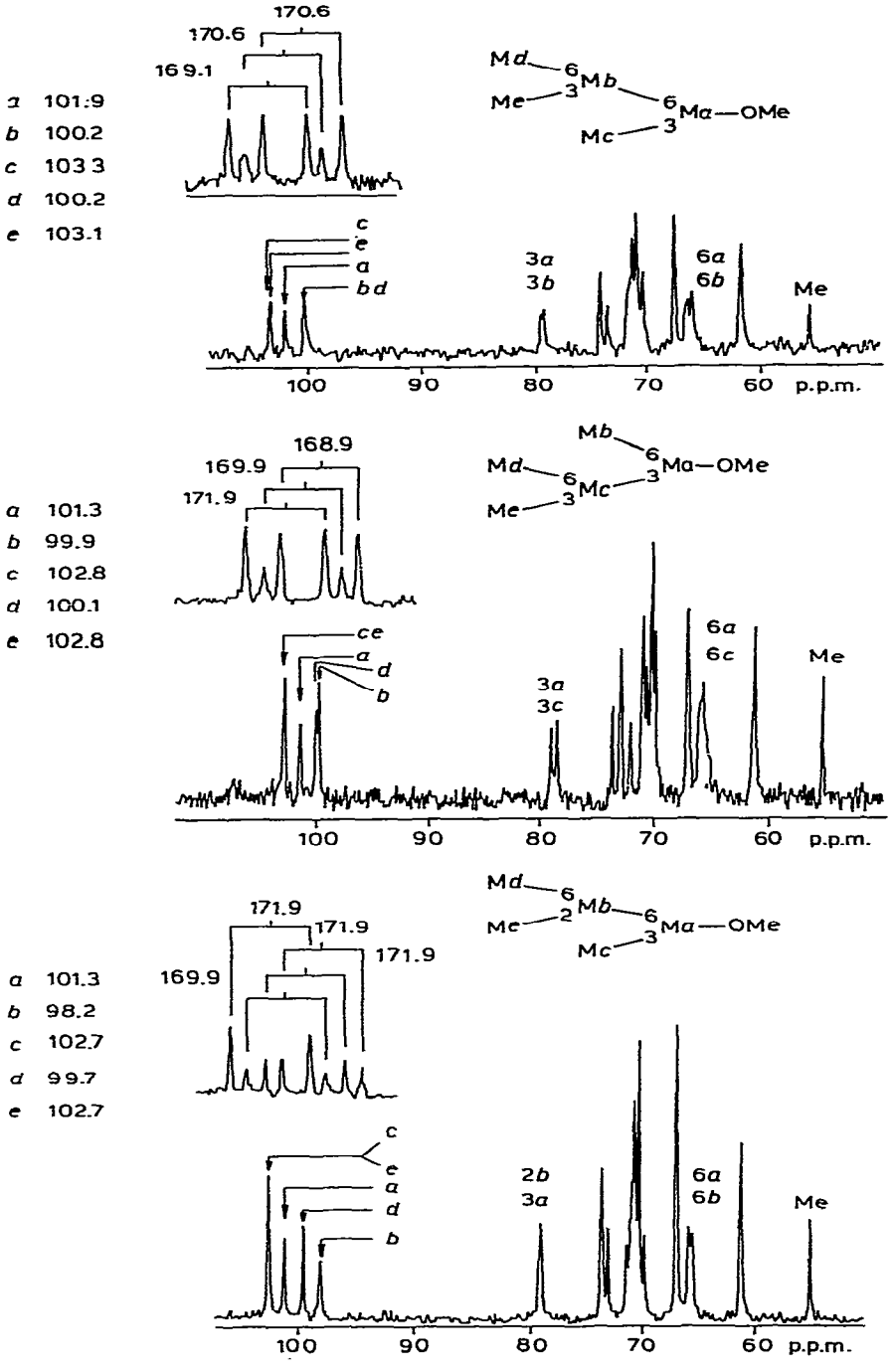


Fig. 9. ^{13}C—N.m.r. spectra of methyl mannopentaosides.

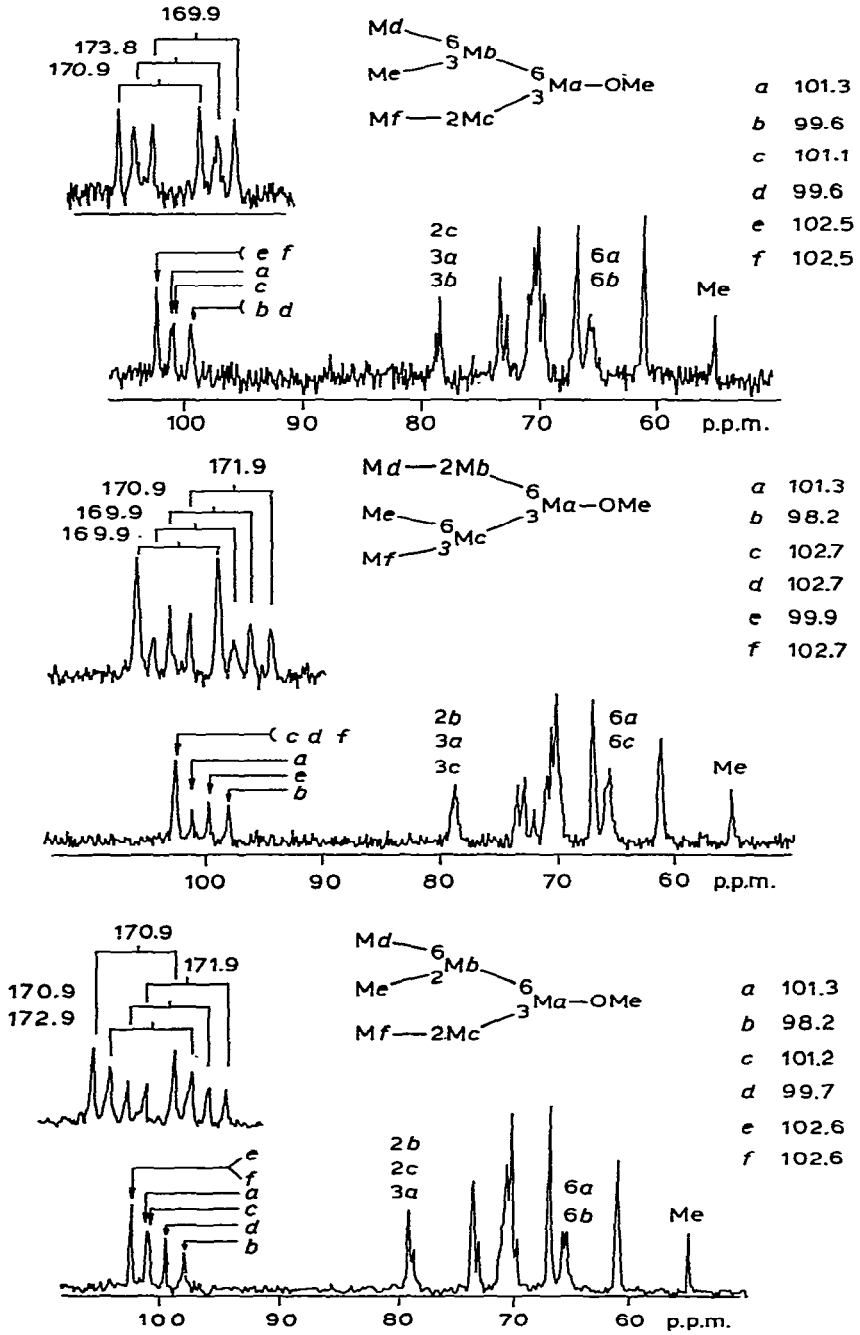


Fig. 10. ^{13}C-N.m.r. spectra of methyl mannohexaosides.

TABLE VIII

OBSERVED AND CALCULATED ^{13}C-CHEMICAL SHIFTS OF THE ANOMERIC CARBON ATOMS FOR INTERNAL MANNOSE RESIDUES (italic *M*)

Structure	*Chemical shift (p.p.m.)*	
	Calculated (glycosidation shift) + (β- and γ-effect)	*Observed*
Ml→2*Ml*→6M[a]	(99.6 — 100.2) — (1.7 — 2.2) = 97.4 — 98.2	98.7(9), 98.3(11), 98.2(16)
Ml→2*Ml*→4M	(101.0 — 101.8) — (1.7 — 2.2) = 98.8 — 100.1	99.4(10)
Ml→2*Ml*→3M	(102.5 — 103.3) — (1.7 — 2.2) = 100.3 — 101.6	101.6(9), 101.1(15), 101.2(17)
Ml→2*Ml*→2M	(102.5 — 103.0) — (1.7 — 2.2) = 100.3 — 101.3	101.0(10), 100.9(11)
Ml ↘ $^{6}_{3}$*Ml*→6M ↗ Ml	(99.6 — 100.2) — (0 — 0.6) = 99.0 — 100.2	100.2(12), 99.6(15)
Ml ↘ $^{6}_{3}$*Ml*→3M ↗ Ml	(102.5 — 103.3) — (0 — 0.6) = 101.9 — 103.3	102.8(13), 102.7(16)
Ml ↘ $^{6}_{2}$*Ml*→6M ↗ Ml	(99.6 — 100.2) — (1.7 — 2.2) = 97.4 — 98.5	98.2(14), 98.2(17)

[a]M represents an α-D-mannopyranosyl residue.

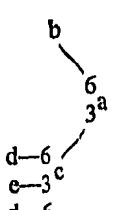
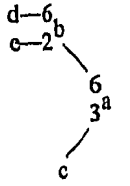
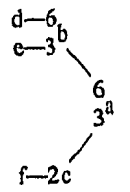
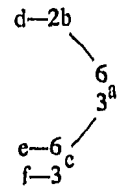
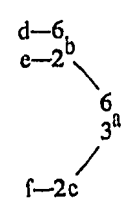
shift and the β- or γ-effect. For example, for the sequence α-D-Man-(1→2)-α-D-*Man*-(1→6)-α-D-Man-, the signal for the anomeric carbon atom of the internal mannose residue should appear at "glycosidation shift" (99.6–100.2) + "β-effect" [—(1.7–2.2)] = 97.4–98.5 p.p.m. Similarly, several signals for the anomeric carbon atoms of internal mannose residues were calculated, as shown in Table VIII. By using the data in Table VIII and empirical rules *ii* and *iii*, signals of the anomeric carbon atoms for methyl mannohexaoside **15**, for example, were assigned as follows. The signal for C-1a was expected to appear at δ 101.0–101.8 p.p.m., and signals for C-1d, C-1e, and C-1f, at δ 99.8–100.3, 102.6–103.2, and 102.6–103.0 p.p.m. From Table IX, signals for C-1b and C-1c were expected to appear at δ 99.0–100.2 and 100.3–101.6 p.p.m. Therefore, the signals observed were assigned as C-1a at δ 101.3, C-1b at 99.6, C-1c at 101.1, C-1d at 99.6, and C-1e and C-1f at 102.5 p.p.m. (The assignments for C-1a and C-1c may have to be interchanged.)

Similarly, all anomeric signals for methyl mannopentaosides and hexaosides

TABLE IX

OBSERVED AND CALCULATED (IN PARENTHESES) ^{13}C-CHEMICAL SHIFTS OF ANOMERIC CARBON ATOMS FOR METHYL MANNOPENTAOSIDES AND METHYL MANNOHEXAOSIDES

Structure	*Chemical shifts (p.p.m.) for each carbon atom*					
	a	*b*	*c*	*d*	*e*	*f*
9 d—2b 6 3[a] e—2c	(101.0–101.8) 101.7	(97.4–98.2) 98.7	(100.3–101.6) 101.6	(102.5–103.0) 103.0	(102.5–103.0) 103.0	
10 d—2b 4 2[a] e—2c	(99.4–100.1) 100.2	(98.8–100.1) 99.4	(100.3–101.3) 101.0	(102.5–103.0) 102.6	(102.5–103.0) 102.6	
11 d—2b 6 2[a] e—2c	(99.8–100.1) 99.7	(97.4–98.2) 98.3	(100.3–101.3) 100.9	(102.5–103.0) 102.6	(102.5–103.0) 102.6	
12 d—6 e—3b 6 3[a] c	(101.0–101.8) 101.9	(99.0–100.2) 100.2	(102.5–103.3) 103.1*[a]	(99.6–100.2) 100.2	(102.5–103.3) 103.3*	

13		(101.0–101.8) 101.3	(99.6–100.2) 100.1*	(101.9–103.3) 102.8	(99.6–100.2) 99.9*	(102.5–103.3) 102.8	
14		(101.0–101.8) 101.3	(97.4–98.5) 98.2	(102.5–103.3) 102.7	(99.6–100.2) 99.7	(102.5–103.0) 102.7	
15		(101.0–101.8) 101.3	(99.0–100.2) 99.6	(100.3–101.6) 101.1	(99.6–100.2) 99.6	(102.5–103.3) 102.5	(102.5–103.0) 102.5
16		(101.0–101.8) 101.3	(97.4–98.2) 98.2	(101.9–103.3) 102.7	(102.5–103.0) 102.7	(99.6–100.2) 99.9	(102.5–103.3) 102.7
17		(101.0–101.8) 101.3	(97.4–98.5) 98.2	(100.3–101.6) 101.2	(99.6–100.2) 99.7	(102.5–103.0) 102.6	(102.5–103.0) 102.6

[a]Assignments described with * may have to be interchanged.

could be assigned. Both the calculated and the observed chemical shifts for anomeric carbon atoms are shown in Table IX.

In conclusion, it was demonstrated that the assignment of signals for the anomeric carbon atoms in the ^{13}C-n.m.r. spectra of branched methyl α-D-manno-oligosaccharides may be deduced by using the data for methyl mannobiosides and methyl mannotriosides as models.

EXPERIMENTAL

N.m.r. spectra (^1H and ^{13}C) were recorded, for solutions in D_2O, with a JNM-FX100FT spectrometer operating at 25.05 MHz. The values of δ_H are expressed in p.p.m. downward from the internal standard, sodium 2,2,3,3-tetradeuterio-4,4-dimethyl-4-silapentanoate. Samples were prepared by "exchanging" them with D_2O, *i.e.* by dissolving them several times in 99.8% D_2O and evaporating *in vacuo*. The values of δ_C are expressed in p.p.m. downward from tetramethylsilane, referenced indirectly with an internal standard of 1,4-dioxane (δ 66.9 p.p.m.).

ACKNOWLEDGMENTS

We thank Dr. J. Uzawa and Mrs. T. Chijimatsu for recording and measuring the n.m.r. spectra, and Dr. H. Homma and his staff for the elemental analyses. We also thank Emeritus Scientist Prof. M. Matsui for his encouragement, and Miss A. Sone for technical assistance.

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