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Note

The equilibrium compositions and conformations of some carbohydrate analogs of *N*-acetylmuramoyl-L-alanyl-D-isoglutamine as determined by ^{1}H-n.m.r. spectroscopy*

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On the basis of the finding that *N*-[2-*O*-(2-acetamido-2,3-dideoxy-D-glucopyranose-3-yl)-D-lactoyl]-L-alanyl-D-isoglutamine (*N*-acetylmuramoyl-L-alanyl-D-isoglutamine; MDP) (**1**) is the minimal, adjuvant-active structure[2,3] capable of replacing whole mycobacterial cells in complete Freund's adjuvant, for (*a*) increasing levels of humoral antibodies against a given antigen, and (*b*) inducing a delayed type of hypersensitivity, a great number of analogs have been synthesized[4] by many groups and their immunological activities investigated[5]. In our continuing efforts to clarify the relationship between the immunoadjuvant activity of, and the structure of the carbohydrate moiety in, MDP, we have shown that change in the configuration of the 2-acetamido-2,3-dideoxy-D-glucose-3-yl group in MDP significantly affects the manifestation of the activity. In fact, the D-*manno* and D-*galacto* analogs of MDP retained the potent adjuvant activity on the induction of delayed-type hypersensitivity to *N*-acetyl-L-tyrosine-3-azobenzene-4′-arsonate in guinea pigs, but the D-*allo*, D-*gulo*, and L-*ido* analogs were all inactive[6,7].

Recently, the anomeric ratio (α:β) of MDP in aqueous solution has been determined[8], by ^{13}C-n.m.r.-spectral measurement and liquid chromatography, to be 2:1. ^{1}H-N.m.r. study[9] on the peptide moiety in MDP has also been reported, but little is known regarding the carbohydrate unit. We now describe the results of 400-MHz, ^{1}H-n.m.r.-spectral studies on the carbohydrate moiety in MDP, and on some of its carbohydrate analogs.

Table I shows the equilibrium compositions of solutions of MDP (**1**), carbohydrate analogs (**3**, **5**, **7**, and **9**), and the corresponding 2-amino- (**4**) and 2-

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*Studies on Immunoadjuvant Active Compounds, Part XXI For Part XX, see ref 1.

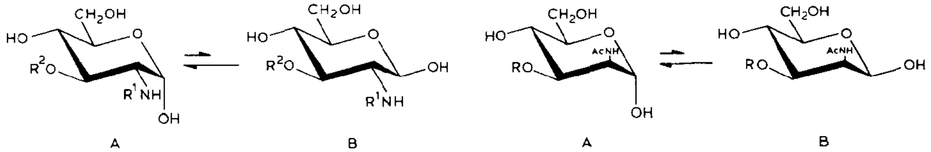
TABLE I

EQUILIBRIUM COMPOSITIONS OF MDP AND SOME OF ITS CARBOHYDRATE ANALOGS IN DEUTERIUM OXIDE, BY ^{1}H-N.M.R. SPECTROSCOPY

Compound	*Equilibrium composition (%)*			
	A *(α-Pyranose)*	*B* *(β-Pyranose)*	*C* *(α-Furanose)*	*D* *(β-Furanose)*
1	66	34	—[a]	—
2	68[b]	32[b]	—	—
3	63	37	—	—
4	63[b]	37[b]	—	—
5	58	42	—	—
6	56	44	—	—
7	10	66	14	10
8	14	72	9	5
9	12	88	—	—
10	17	74	3	6

[a] Not detected. [b] Data from ref. 10.

acetamido-2-deoxy-D-hexoses (**2**, **6**, **8**, and **10**). The α:β ratio for MDP (**1**) was estimated at 2:1, as reported by Halls *et al.*[8], and a similar ratio was also observed for *N*-[2-*O*-(2-amino-2,3-dideoxy-D-glucopyranose-3-yl)-D-lactoyl]-L-alanyl-D-isoglutamine[12] (**3**). *N*-[2-*O*-(2-Acetamido-2,3-dideoxy-D-mannopyranose-3-yl)-D-lactoyl]-L-alanyl-D-isoglutamine[6] (**5**) had a ratio of 29:21. These anomeric compositions of solutions of **1**, **3**, and **5** were almost the same as those for the corresponding 2-acetamido-2-deoxy-D-glucose (**2**), 2-amino-2-deoxy-D-glucose (**4**), and 2-acetamido-2-deoxy-D-mannose (**6**); see Scheme 1. In the case of *N*-[2-*O*-(2-acetamido-2,3-dideoxy-D-allose-3-yl)-D-lactoyl]-L-alanyl-D-isoglutamine[6] (**7**), pyranose–furanose equilibrium (in addition to α:β anomeric equilibrium) was observed, and the furanose composition of a solution of **7** was richer than that of 2-acetamido-2-deoxy-D-allose[13] (**8**).



$1\ R^1 = Ac,\ R^2 = a$

$2\ R^1 = Ac,\ R^2 = H$

$3\ R^1 = H, R^2 = a$

$4\ R^1 = H, R^2 = H$

$Ac = CH_3CO$

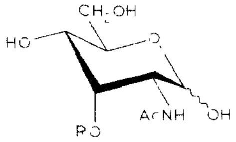
a = −CH(Me)CO−L-Ala−D-isoGln

$5\ R = a$

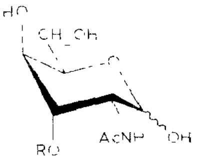
$6\ R = H$

Scheme 1

On the other hand, for *N*-[2-*O*-(2-acetamido-2,3-dideoxy-D-gulose-3-yl)-D-lactoyl]-L-alanyl-D-isoglutamine[6] (**9**), the furanose forms were not detected, although a solution of the corresponding 2-acetamido-2-deoxy-D-gulose[14] (**10**) contains appreciable proportions of furanoses. The major equilibrium compositions of **7**–**10** are the β-D-pyranose forms, which are disfavored for **1**–**6**. It is known that solutions of D-allose and D-gulose contain appreciable proportions of furanose forms at equilibrium, and that the major tautomer of these sugars is the β-D-pyranose form[11]. As shown in Table I, introduction of the lactoyldipeptide group at O-3 of **8**, to give **7**, increased the population of furanose forms, whereas the same operation for **10**, to give **9**, decreased the furanose forms. This result indicates that steric hindrance between the lactoyldipeptide moiety and the 5- and 6-hydroxyl groups in the furanose forms of **9** may be responsible for decreasing the furanose forms.



7 R = a
8 R = H



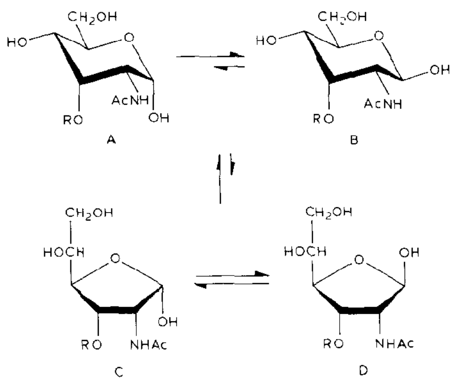
9 R = a
10 R = H

Tables II and III respectively show the chemical shifts and the coupling constants for the carbohydrate group in **1**, **3**, **5**, **7**, and **9**, and these values are very similar to those of the corresponding 2-amino- and 2-acetamido-2-deoxy-D-hexoses. It was reported that the chair form favored by the various aldohexopyranoses appears to be controlled by the tendency of the 5-(hydroxymethyl) group to assume the equatorial orientation, and that D-mannose, D-allose and D-gulose, as well as D-glucose, favor the $^4C_1$(D) conformation[11]. The coupling constants (see Table III) indicate that the pyranose forms of compounds **1**, **3**, **5**, **7**, and **9** also exist preponderantly in the $^4C_1$(D) conformation, and, consequently, that the lactoyldipeptide group in **7α,β** and **9α,β** is axial.

The immunoadjuvant activities of **7** and **9** on the induction of delayed-type hypersensitivity in guinea pigs were negative, whereas compounds **1**, **3**, **5**, and the D-*galacto* analog are active as adjuvants[6,7]. This suggests that, for manifestation of the activity, the lactoyldipeptide group on O-3 of the sugar should be equatorially attached.

EXPERIMENTAL

^{1}H-N.m.r. spectra. — A solution (1%) of each compound in deuterium oxide (99.75%) containing a trace of sodium 4,4-dimethyl-4-silapentane-1-sulfonate (DSS) as the internal standard was kept for more than 24 h. The spectra were recorded at room temperature (23–25°) with a JEOL FX-400 spectrometer operated at 400 MHz in the Fourier-transform mode.



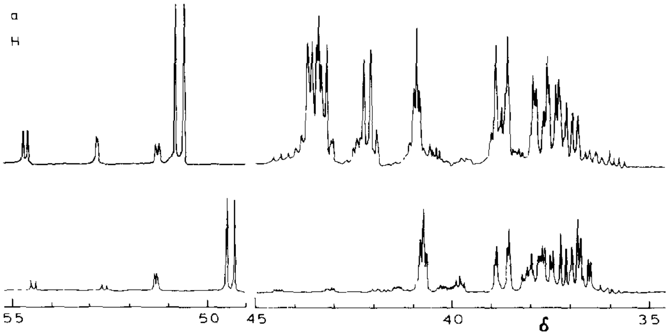


Fig. 1. Partial, ^{1}H-n.m.r. spectra of 7 (upper trace) and 8 (lower trace).

TABLE II

CHEMICAL SHIFTS OF RING PROTONS IN MDP AND SOME OF ITS CARBOHYDRATE ANALOGS IN DEUTERIUM OXIDE

Compound	*Chemical shifts (δ from DSS[a])*							
	H-1	*H-2*	*H-3*	*H-4*	*H-5*	*H-6*	*H-6'*	*AcN*
1A	5.16	3.95	3.70	3.57	3.87	3.80	3.84	1.97
1B	4.67	3.78	3.55	3.52	3.47	3.76	3.90	1.96
3A	5.41	3.30	3.76	3.61	3.88	3.77	3.83	—
3B	4.93	3.01	3.68	3.62	3.50	3.73	3.89	—
5A	5.16	4.44	3.89	3.70	3.82	3.87	3.88	2.05
5B	5.01	4.55	3.74	3.62	3.43	3.82	3.89	2.08
7A	5.13	4.08	4.11	3.81	4.00	3.78	3.87	2.08
7B	5.07	3.75	4.09	3.78	3.85	3.70	3.87	2.05
7C	5.46	4.39			←4.40–3.56→			2.09
7D	5.28				←4.40–3.56→			2.08
9A	5.16	4.36	3.81	3.92	4.23	3.72	3.72	2.07
9B	4.98	4.03	3.79	3.78	4.00	3.75	3.69	2.05

[a] DSS: sodium 4,4-dimethyl-4-silapentane-1-sulfonate.

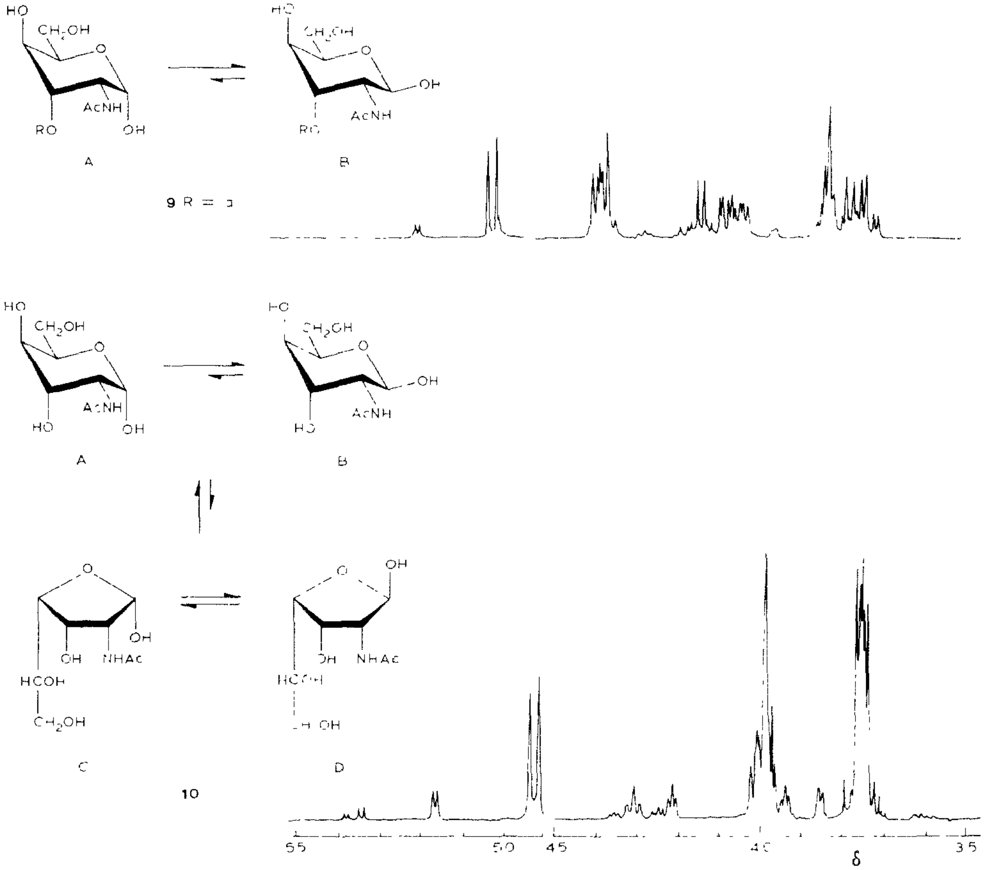


Fig 2. Partial, ^{1}H-n.m.r. spectra of **9** (upper trace) and **10** (lower trace)

TABLE III

COUPLING CONSTANTS OF RING PROTONS IN MDP AND SOME OF ITS CARBOHYDRATE ANALOGS IN DEUTERIUM OXIDE

Compound	*Coupling constants (Hz)*						
	$J_{1,2}$	$J_{2,3}$	$J_{3,4}$	$J_{4,5}$	$J_{5,6}$	$J_{5,6'}$	$J_{6,6'}$
1A	3.4	10.3	9.3	9.8	4.9	2.4	12.2
1B	8.3	10.3	9.3	10.3	5.4	2.0	12.2
3A	3.4	10.3	9.3	10.3	4.9	2.0	12.2
3B	8.3	10.3	8.8	9.3	5.4	2.0	12.2
5A	1.5	4.4	9.3	9.8	4.4	2.0	12.2
5B	1.5	3.9	9.3	9.8	4.4	2.0	12.2
7A	3.9	2.9	2.9	10.3	5.4	2.0	12.2
7B	8.8	2.9	2.9	9.8	5.4	2.0	12.2
7C	4.9						
7D	2.0						
9A	3.9	3.9	3.9	1.0	6.0	6.0	—[a]
9B	8.8	3.4	3.4	0.5	7.8	4.4	11.7

[a] Geminal coupling was not observed.

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