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Note

^{13}C-N.m.r studies of a natural immunoadjuvant, peptidoglycan monomer and related compounds

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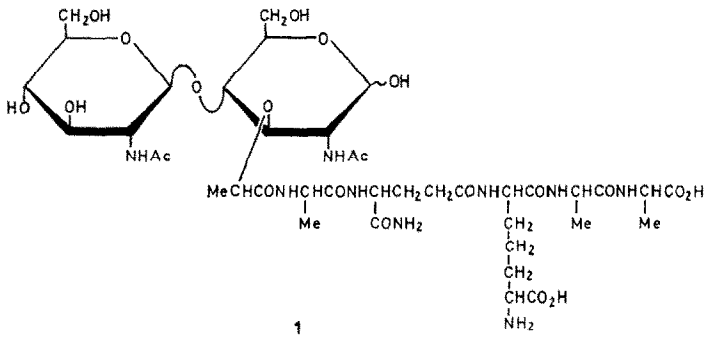
Peptidoglycans, which are common constituents of bacterial cell-walls, elicit a variety of biological responses in non-bacterial systems[1]. The natural biopolymer and synthetic analogues of low molecular weight exhibit marked immunomodulating properties[2–5].

The peptidoglycan monomer used in this work and characterised[6] as [2-acetamido-4-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-2-deoxy-3-*O*-(D-ethyl-1-carbonyl)-D-glucopyranose]-L-alanyl-D-isoglutaminyl-[(L)-*meso*-diaminopimeloyl-(L)-D-alanyl-D-alanine] (**1**) was obtained by lysozyme digestion of linear, non-cross-linked, peptidoglycan polymer-chains isolated from culture fluids of penicillin-treated *Brevibacterium divaricatum*[6,7].



1

L-Alanyl-D-isoglutaminyl-[(L)-*meso*-diaminopimeloyl-(L)-D-alanyl-D-alanine] (**2**) and 2-acetamido-4-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-3-*O*-[1-(*S*)-carboxyethyl]-2-deoxy-D-glucopyranose (**3**) were obtained from **1** by hydrolysis with *N*-acetylmuramoyl-L-alanine amidase[8].

A preliminary ^{13}C-n.m.r. investigation of an *N*-acetylmuramoyl-dipeptide has appeared[9], but there are no data on larger peptidoglycan fragments. We now report

on the assignment of the ^{13}C resonances for **1–3**. The relevant data are included in Table I, together with those for the model compounds D,L-diaminopimelic acid (**4**), 2-acetamido-2-deoxy-D-glucopyranose (**5**), 2-acetamido-3-*O*-[1-(*S*)-carboxyethyl]-2-deoxy-D-glucopyranose (**6**, *N*-acetylmuramic acid), *N*-acetylmuramoyl-L-alanine (**7**), *N*-acetylmuramoyl-L-alanyl-D-glutamic acid (**8**), and *N*-acetylmuramoyl-L-alanyl-D-isoglutamine (**9**).

The data for **5** accord with literature values[9,11], and comparison with those for **6** revealed a downfield shift for the C-3 signal of **6**, with respect to that of **5** (8.8 and 8.9 p.p.m. for the α and β anomer, respectively), due to the carboxyethyl substituent.

For the disaccharide **3**, the signals of the carbons involved in the linkage were shifted to lower field, compared with those for the constituent monosaccharides, *i.e.*, 6.4 p.p.m. for C-4 of *N*-acetylmuramic acid, and 5.3 p.p.m. for C-1 of 2-acetamido-2-deoxy-D-glucopyranose. Similar shielding effects in disaccharides have been reported for α-cellobiose[12] and for 6-aminohexyl glycosides of *O*-β-D-galactopyranosyl-2-acetamido-2-deoxy-β-D-glucopyranose[13]. A downfield shift (2.1 p.p.m.) with respect to free **6** was also observed for the carboxyl signal of the *N*-acetylmuramic acid moiety in **3**. The chemical shifts of C-2,3,5 in this moiety suggest the preponderance of the α configuration; on the basis of signal intensities, an $\alpha\beta$-ratio of 3:1 was determined.

The assignment of the signals of the pentapeptide **2** was based on the data available for **4**, and on literature data[14,15] for free and peptide-bound alanine, glutamic acid, and isoglutamine.

Whereas **4** gave only four ^{13}C resonances, the signals of all the seven carbons were resolved in the α,α-diaminopimeloyl moiety of **2**, which is non-symmetrically substituted at the L-chiral centre only. This substitution caused upfield shifts for C-α (1.6 p.p.m.), C-α' (unsubstituted, 1.5 p.p.m.), and α-CO (1.0 p.p.m.), but a downfield shift (0.3 p.p.m.) for α'-CO.

In comparison with the free amino acid, the alanine residues in **2** showed downfield shifts for the signals for C-α (1.2 p.p.m. for *C*-terminal, and 0.6 p.p.m. for the other two alanine residues), and upfield shifts for C-β (1.0 p.p.m. for *C*-terminal, and 1.3 p.p.m. for the other two alanine residues). The carbonyl signals were shifted 0.9 p.p.m. for *N*-terminal alanine and 2.2 p.p.m. for peptide-bound alanine, both to higher field; that of *C*-terminal alanine remained unchanged.

Comparison of the ^{13}C data for **6** with those for synthetic *N*-acetylmuramoyl-L-alanine (**7**), *N*-acetylmuramoyl-L-alanyl-D-glutamic acid[9] (**8**), and *N*-acetylmuramoyl-L-alanyl-D-isoglutamine[9] (**9**) revealed that the lactylamide bond affected the signal of the carboxyethyl residue: upfield shifts for the carbonyl (5.15 and 4.7 p.p.m. for α- and β-D-*N*-acetylmuramoyl, respectively), and downfield shifts for the C-α signals (1.0 and 1.3 p.p.m.) for α- and β-D-*N*-acetylmuramoyl, respectively).

The ^{13}C resonances associated with the pentapeptide moiety in **1** were not more than 0.5 p.p.m. apart from those in free **2**. In both **1** and **2**, the second member of the peptide chain was shown to be isoglutamine by comparing the respective C-γ and δ-carbonyl resonances with those for synthetic **8** and **9**.

TABLE I

^{13}C-N.M.R. DATA FOR COMPOUNDS **1–9**[a]

Assignment	*Compound*													
	1	**2**	**3**	**4**	**5α**	**5β**	**6α**	**6β**	**7α**	**7β**	**8α**	**8β**	**9α**	**9β**
2-Acetamido-2-deoxy-D-glucosyl residue														
C-1	100.4		100.3		90.9	95.0								
C-2	56.05		56.3		54.1	56.8								
C-3	73.6		73.6		70.8	73.9								
C-4	70.45		70.5		70.2	70.0								
C-5	75.35		75.7		71.6	76.0								
C-6	61.25		61.4		60.7	60.7								
CH_3CO	173.5[b]		174.5[d]		174.4	174.7								
CH_3CO	22.3		22.1		22.0	22.3								
N-*Acetylmuramoyl residue*														
C-1	β 95.1		β 95.4				90.8	94.9	91.0	94.95	91.0	94.95	91.0	94.9
	α 90.2		α 89.9											
C-2	53.7		54.7				53.6	57.85	53.7	57.85	53.7	56.15	53.7	56.1
C-3	79.55		78.9				79.6	82.8	79.6	82.5	79.6	82.45	79.55	82.5
C-4	76.35		76.3				69.9	69.9	69.1	68.9	69.1	68.8	69.0	68.9
C-5	71.2		71.3				71.7	75.8	71.6	75.8	71.5	75.8	71.5	75.75
C-6	59.9		59.7				60.6	60.6	60.8	60.8	60.55	60.7	60.55	60.7
CH_3CO	174.0		174.4[d]				174.2	174.2	173.9	174.2	173.9	174.2	173.9	174.2
CH_3CO	22.2		22.1				22.1	22.1	22.1	22.3	22.0	22.3	22.0	22.4
CH	77.45		77.4				76.9	76.9	77.9	78.2	77.75	78.1	77.7	78.0
CH_3	18.4		18.5				18.5	18.5	18.6	18.6	18.7	18.7	18.65	18.65
CO	174.9[c]		182.1				180.0	180.0	174.85	175.3	174.8	175.4	174.85	175.75[f]

L-*Alanyl residue*										
CHα	49.6	49.2		49.6	49.6	49.6	49.6	49.65	49.65
CH$_3\beta$	16.8	16.8		16.5	16.5	16.9	16.9	16.5	16.5
CO	174.6[e]	174.8		175.6	175.6	175.7	175.7	175.8[e]	175.8[f]
D-*Glutamyl or* D-*isoglutaminyl residue*									
CO	175.2[e]	173.5				174.8	174.8	175.8[e]	175.8[f]
CHα	53.7	53.7				53.6	53.6	52.7	52.7
CH$_2\beta$	27.15	27.0				26.0	26.0	26.3	26.3
CH$_2\gamma$	31.55	31.3				30.1	30.1	31.1	31.1
COδ	175.7	175.5				177.0	177.0	175.85[e]	175.85[f]
D,L-*Diaminopimeloyl*									
CO	173.5[b]	173.5	174.5						
CHα	52.75	52.9	54.5						
CH$_2\beta$	30.6	30.4	30.25						
CH$_2\gamma$	20.75	20.7	20.8						
CH$_2\beta'$	30.7	30.5	30.25						
CHα'	52.75	53.0	54.5						
CO	174.9[e]	174.8	174.5						
D-*Alanyl residue*									
CHα	49.6	49.2							
CH$_3\beta$	16.75	16.8							
CO	173.4[b]	173.5							
D-*Alanyl residue*									
CHα	49.9	49.5							
CH$_3\beta$	17.7	17.4							
CO	175.7[e]	175.5							

[a]Chemical shifts (δ values) in p.p.m. from tetramethylsilane; δ (Me$_4$Si) = δ (1,4-dioxane) + 66.6. [b–f]Assignments may be interchanged.

An $\alpha\beta$-ratio of 2:1 was indicated for the muramoyl residue of **1**, which accords with literature reports[9] for *N*-acetylmuramoyl-dipeptides.

Thus, the ^{13}C-n.m.r. data for **1** corroborate the structure determined by chemical methods[1,6], particularly with regard to the postulated (1→4)-β-D linkage between 2-acetamido-2-deoxy-D-glucopyranose and *N*-acetylmuramic acid. This linkage has also been found in naturally occurring, polymeric peptidoglycans susceptible to lysozyme hydrolysis. The configuration at the reducing end of **1** and **3** (mainly α), tentatively assigned on the basis of the present data, may arise by mutarotation following enzymic action.

EXPERIMENTAL

^{13}C-N.m.r. spectra were recorded with a JEOL FX 90 Q Fourier-transform spectrometer operating at 22.5 MHz for 0.1M solutions in 99.75% D_2O at room temperature in 5-mm o.d. tubes. The sweep width used was 5200 Hz, the pulse width was 5 μs (90° pulse), the acquisition time was 2 s, and the digital resolution was 0.056 p.p.m. Chemical shifts were measured (accuracy of the chemical shift, ±0.1 p.p.m.) relative to that of internal 1,4-dioxane, set at 66.6 p.p.m. downfield of that of Me_4Si.

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