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Pullulan nonaacetate: Assignment of chemical shifts of the acetyl protons and acetyl carbonyl carbons by 2D-NMR spectroscopy [1]

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Abstract

Pullulan nonaacetate, a homogulucan having an α-(1 → 6)-linked maltotriosyl repeating-unit, was studied by ^{1}H and ^{13}C NMR spectroscopy. Nine resolved signals were observed for both the methyl protons and for the carbonyl carbons of the acetyl substituents. A series of 2D-NMR experiments was used to assign fully the proton and carbon chemical shifts of pullulan nonaacetate. The determination of long-range correlations between acetyl protons and maltotriosyl carbons, and between acetyl carbonyl carbons and maltotriosyl protons, by a field-gradient HMBC (heteronuclear multibond coherence) experiment was of particular utility for assigning the protons and the carbons of the acetyl groups. © 1998 Elsevier Science Ltd

Keywords: Pullulan nonaacetate; Polysaccharide derivatives; NMR structural study

1. Introduction

Pullulan, a linear polysaccharide having an α-(1 → 6)-linked maltotriosyl repeating unit, has now become commercially available [1]. A variety of pullulan derivatives have been prepared to examine their potential applications in the food, cosmetics, pharmaceutics and electronics fields [1–4].

Chemical modification of pullulan may be performed, as with cellulose, by esterification or etherification of hydroxyl groups in a maltotriosyl unit. As the maltotriosyl unit contains nine hydroxyl groups in a geometrically unique environment, the structural diversity of pullulan derivatives surpasses that in cellulose or other polysaccharides having a single glycosyl repeating unit. Since product liability is a persistent demand for pullulan derivatives in their potential fields of application, an analytical means for providing detailed structural information [5–9] is of critical importance for achieving rigorous quality control in production processes.

We have recently proposed a new analytical technique for cellulose and other polysaccharide derivatives, in particular ester and ether derivatives, in which unsubstituted hydroxyl groups in the starting polysaccharide derivative are peracylated (mostly

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[1] Part 12 of a series;. ^{1}H and ^{13}C NMR Structural Studies on Cellulose and Polysaccharide Derivatives with Carbonyl Groups as a Sensitive Probe. For preceding papers, see ref. [17].

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acetylated) and examined by ^{13}C NMR [10–17]. It has been demonstrated that acetyl or other acyl carbonyl carbon signals can be utilized as a remarkably sensitive structural probe for providing detailed structural information, including the distribution of acetyl or acyl substituents within a glycosyl unit in polysaccharide derivatives.

Extending the preceding studies, we report here on the full assignment of nine peaks observed both in the acetyl proton and acetyl carbonyl carbon signals in pullulan peracetate. A series 2D-NMR techniques, including H–H DQF–COSY and H–H NOESY techniques, and in particular a field-gradient HMBC technique [18], permitted direct assignment of all relevant peaks.

2. Experimental

Samples.—Pullulan (PF-20), having a viscosity value of 7720 centipoises in 20% aqueous solution at 20 °C, was supplied by the Shin–Etsu Chemical Co. The purity of the sample was confirmed by ^{1}H and ^{13}C NMR spectroscopic analysis and it was used without further purification. Pullulan was peracetylated as follows: in a 200-mL two-neck flask, pullulan (2.0 g, 1.25×10^{-2} mol by glucosyl unit), pyridine (40 mL) and 4-(dimethylamino)pyridine (0.5 g) were introduced. After heating at 100 °C, Ac_2O (20 mL) was added, and heating was continued for 2 h under stirring. The product was recovered by pouring the mixture into water, and was subsequently purified by reprecipitation from an acetone–water system. The recovered product was dried under diminished pressure, and finally freeze-dried from benzene in order to remove residual water in the sample. A white powdery product (3.19 g) was thus obtained.

Measurements.—^{1}H and ^{13}C NMR measurements were performed with a J EX-400, or with a J LA-400 for HMBC measurements, spectrometer at 400 MHz for ^{1}H and 100 MHz for ^{13}C, respectively, with a 5-mm diameter C–H dual probe at 40 °C. The sample concentration was 10 w/vol%. Chemical shift values were referenced from the residual proton signal of $CDCl_3$ (7.30 ppm) or from the triplet middle carbon signal of $CDCl_3$ (77.0 ppm).

The H–H correlated 2D spectroscopy (DQF–COSY) measurements were performed with a 1024 × 256 data matrix, and four transients were acquired for each t_1 value. A spectral width of 2362 Hz was used in both dimensions and the pulse repetition was set to 1.5 s. The spectrum was then subjected to FT-processing after zero-filling to a 1024 × 512 matrix.

The H–H NOESY measurements were performed by a phase-sensitive mode with a 1024 × 256 data matrix. A mixing time of 200 ms was chosen and four transients were acquired for each t_1 value. A spectral width of 2370 Hz was used in both dimensions and the pulse repetition was set to 3.0 s. The spectrum was then subjected to FT-processing after zero-filling to a 1024 × 512 matrix.

The C–H correlated 2D spectroscopy (C–H COSY) measurements were conducted with a routine shift-correlation mode with a 2048 × 256 data matrix. The

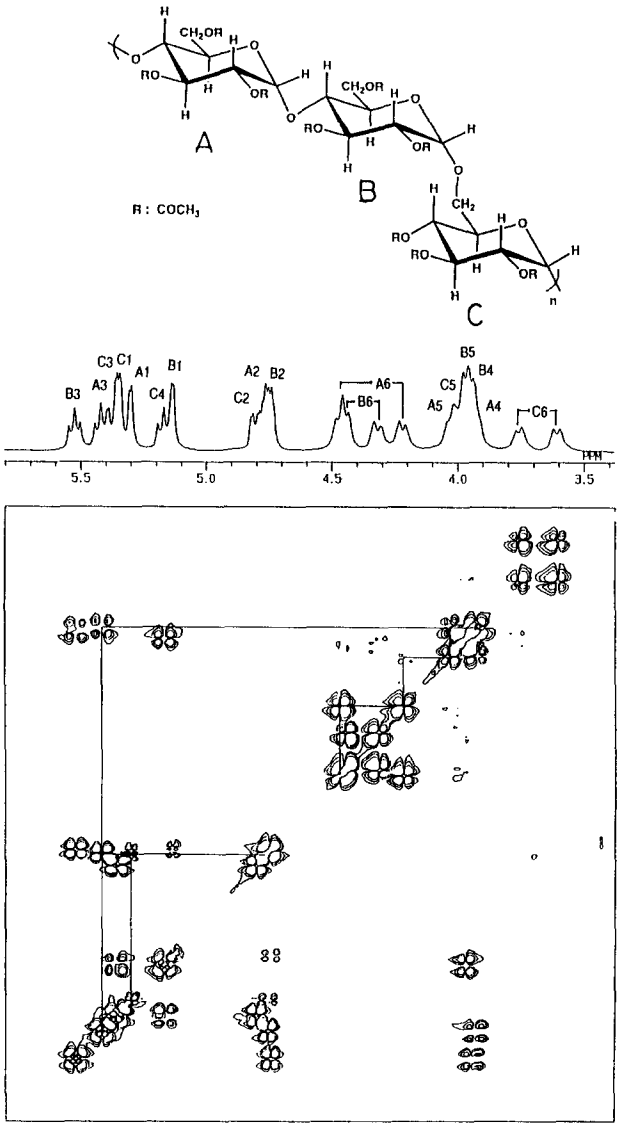

Fig. 1. Maltotriosyl region of the 400 MHz H–H DQF–COSY spectrum of pullulan peracetate in $CDCl_3$ at 40 °C. The H–H connectivities in the glucose residue A are indicated.

Table 1
Assignments of proton and carbon signals in pullulan peracetate

Position[a]	Maltotriose protons	Maltotriose carbons	Acetyl protons	Acetyl carbonyl carbons
A1	5.26	95.62	–	–
A2	4.71[b]	70.58	2.02	170.72
A3	5.38	71.89	1.99	169.64
A4	3.88[b]	72.76	–	–
A5	3.97[b]	69.00	–	–
A6	4.17, 4.43	62.84	2.13	170.32
B1	5.10	95.99	–	–
B2	4.70[b]	71.32	2.07	170.45
B3	5.49	72.30	1.96	169.42
B4	3.90[b]	73.88	–	–
B5	3.92[b]	69.77	–	–
B6	4.28, 4.41	63.10	2.14	170.34
C1	5.31	95.60	–	–
C2	4.76[b]	70.10	2.01	170.52
C3	5.32	69.79	1.96	169.85
C4	5.13	68.39	2.05	169.02
C5	3.94[b]	68.03	–	–
C6	3.57, 3.72	64.80	–	–

[a]Three glucosyl groups in the maltotriose unit are denoted as A, B, and C; 4)-α-D-Glc^{A}-(1 → 4)-α-D-Glc^{B}-(1 → 6)-α-D-Glc^{C}-(1 → . See also the structural formula given in Fig. 1.
[b]Estimated from the DQF–COSY spectrum given in Fig. 1.

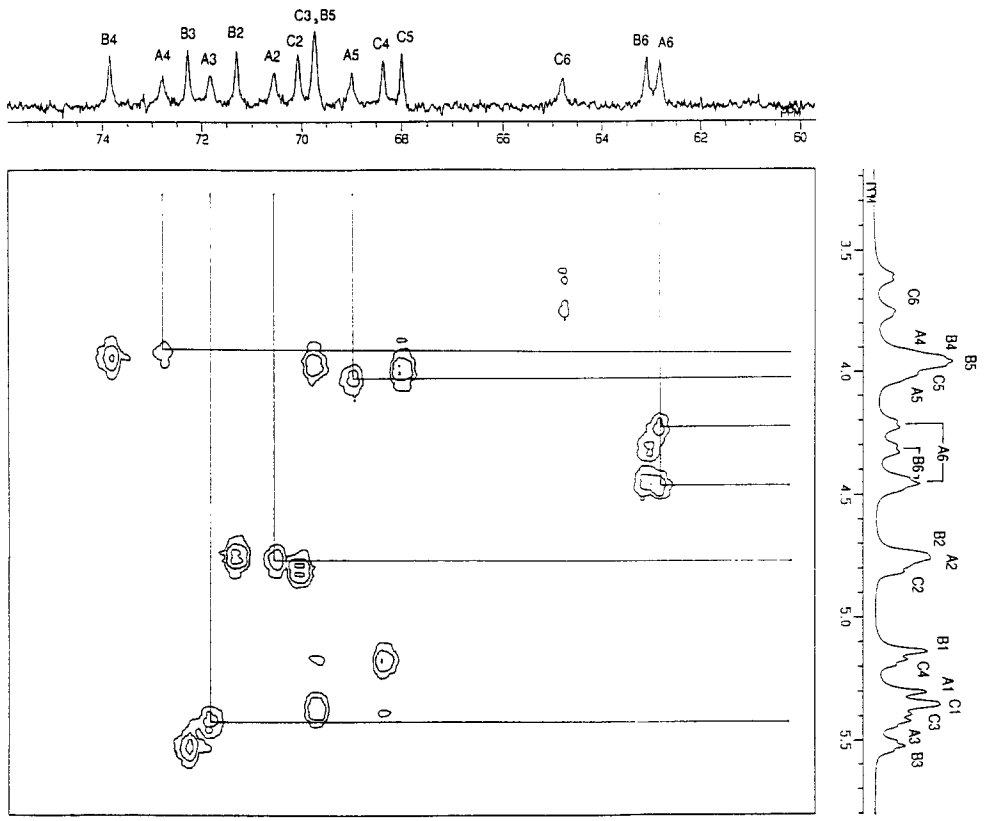

Fig. 2. Maltotriosyl region of the 400 MHz C–H COSY spectrum of pullulan peracetate in $CDCl_3$ at 40 °C. The C–H connectivities in the glucose residue A are indicated. For the glucosyl unit A, B, and C; 4)-α-D-Glc^{A}-(1 → 4)-α-D-Glc^{B}-(1 → 6)-α-D-Glc^{C}-(1 → , see also the structural formula given in Fig. 1.

spectral width for ^{13}C and ^{1}H dimensions were 17 921 and 2416 Hz, respectively, and 256 transients were acquired with a pulse delay of 3.0 s. The spectrum was then subjected to FT-processing after zero-filling to a 2048 × 512 matrix.

Multiple-bond (long-range) C–H correlated 2D spectroscopy (long-range C–H COSY) measurements were performed by a field-gradient mode HMBC (heteronuclear multibond coherence) technique. A total of 512 experiments of 2048 data points, consisting of 256 transients acquired for each t_1 value, were recorded after applying 16 dummy scans. Spectral widths for ^{13}C and ^{1}H dimensions were 18 051 and 2435 Hz, respectively, and the pulse repetition was set to 1.7 s. The delays for coherence transfer were set to 3.4 and 60 ms, respectively. The spectrum was then subjected to FT-processing after zero-filling to a 2048 × 1024 matrix. The total measurement time was 65 h.

3. Results and discussion

^{1}H and ^{13}C NMR measurements of cellulose and other peracetylated polysaccharide derivatives have shown that both acetyl proton and acetyl carbonyl carbon signals are sensitive to their location in the glycose residues. For instance, resolved triplets are observed for cellulose and amylose triacetates, and these are indicative of substitution positions in the glucose residues [19].

This remarkably sensitive nature of acetyl group signals in the NMR analysis of polysaccharide derivatives has been applied for determining the substituent distribution in partly substituted cellulose esters and ethers, through acetylation (or the appropriate acylation in some instances) of unsubstituted hydroxyl groups in the glucose residues [10–17]. Furthermore, acetyl proton signals in cellulose and amylose triacetates have been shown to be a versatile

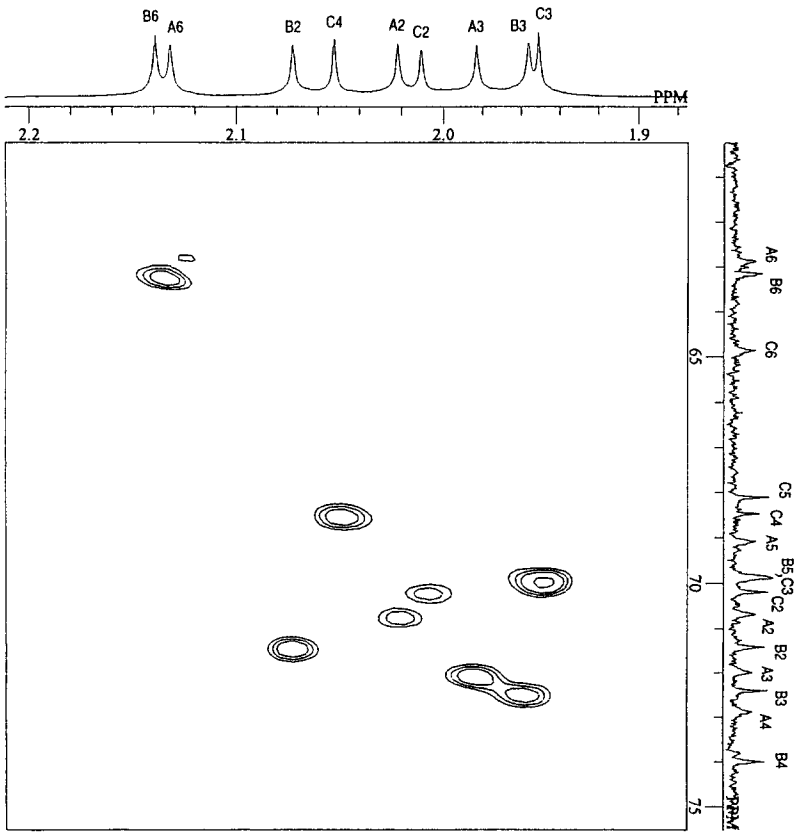

Fig. 3. The 400 MHz HMBC spectrum of pullulan peracetate in $CDCl_3$ at 40 °C, displaying connectivity between acetyl protons and maltotriosyl carbons. For the glucose residues unit A, B, and C; 4)-α-D-Glc^{A}-(1 → 4)-α-D-Glc^{B}-(1 → 6)-α-D-Glc^{C}-(1 → , see also the structural formula given in Fig. 1.

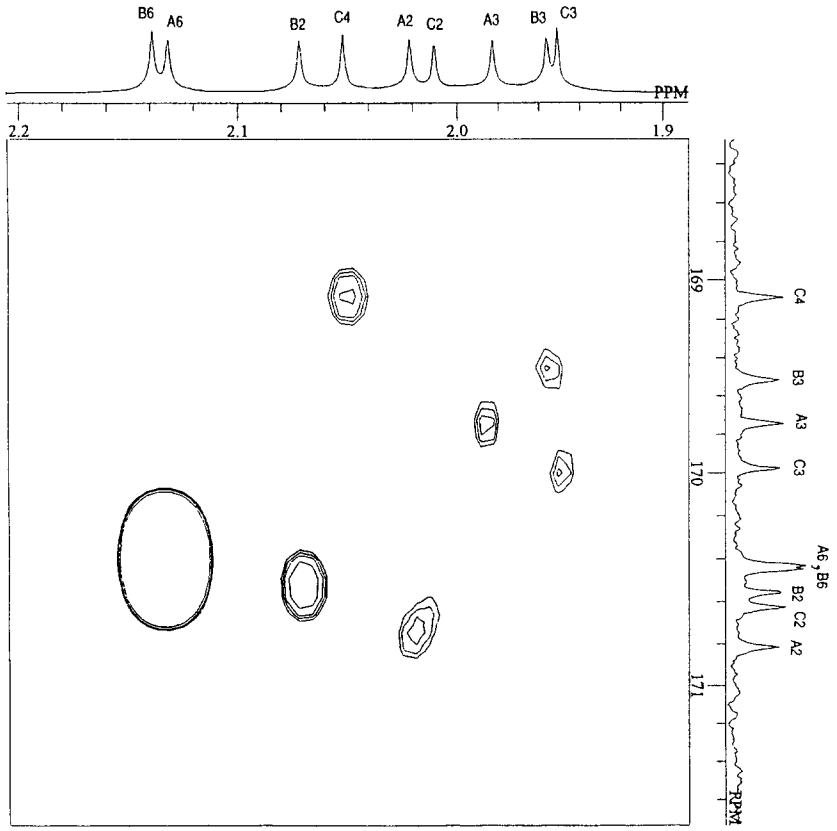

Fig. 4. The 400 MHz HMBC spectrum of pullulan peracetate in $CDCl_3$ at 40 °C, displaying connectivity between acetyl protons and acetyl carbonyl carbons. For the glucosyl unit A, B, and C; 4)-α-D-Glc^A-(1 → 4)-α-D-Glc^B-(1 → 6)-α-D-Glc^C-(1 → , see also the structural formula given in Fig. 1.

structural probe for elucidating static and dynamic super-structures in solution via NOESY measurements. Through-space interactions can be detected between acetyl groups located at different substitution positions [19].

Extending the preceding studies, the present paper describes the complete assignment of nine signals observed for acetyl proton and acetyl carbonyl carbon in pullulan peracetate, by means of a series of 2D-NMR techniques.

Maltotriosyl protons and carbons assignment.—An H–H COSY measurement was performed to observe three-sets of connectivity of proton signals within each glucosyl group in the maltotriosyl unit. A DQF–COSY measurement (Fig. 1) shows sufficiently resolved off-diagonal correlation peaks, even at very crowded peak regions in (4.7–4.8 and 3.9–4.1 ppm). The chemical-shift value of each signal was thus unambiguously determined, leading to the full assignment of the three sets of proton signals in the maltotriosyl unit.

The connectivity between each glucose residue (shown as A, B, and C in Fig. 1) was determined by a phase-sensitive mode NOESY measurement (not shown) with a mixing time of 200 ms. Off-diagonal correlation peaks due to adjacent glucose residues were visible between proton signals at the A1-B4, B1-C6, and C1-A4 positions, respectively, as well as those between protons within each glucose residue. Thus assignment of all proton signals in the maltotriosyl unit has been completed, and the data are collected in Table 1 (first column).

The assignment of carbon signals in the maltotriosyl unit was subsequently performed by C–H COSY measurements as shown in Fig. 2 (the C-1 region is not shown). A series of resolved correlation peaks between each proton signal and the corresponding carbon signals is observed, allowing the full assignment of carbon signals in the maltotriosyl unit, as summarized in Table 1 (second column).

Acetyl protons and acetyl carbonyl carbons assignment.—In preceding reports [15,19], the assignment of acetyl and other acyl carbonyl carbon signals in perester derivatives of cellulose and amylose was accomplished by a 1D INAPT (selective INEPT) technique. Thus a long-range polarization transfer

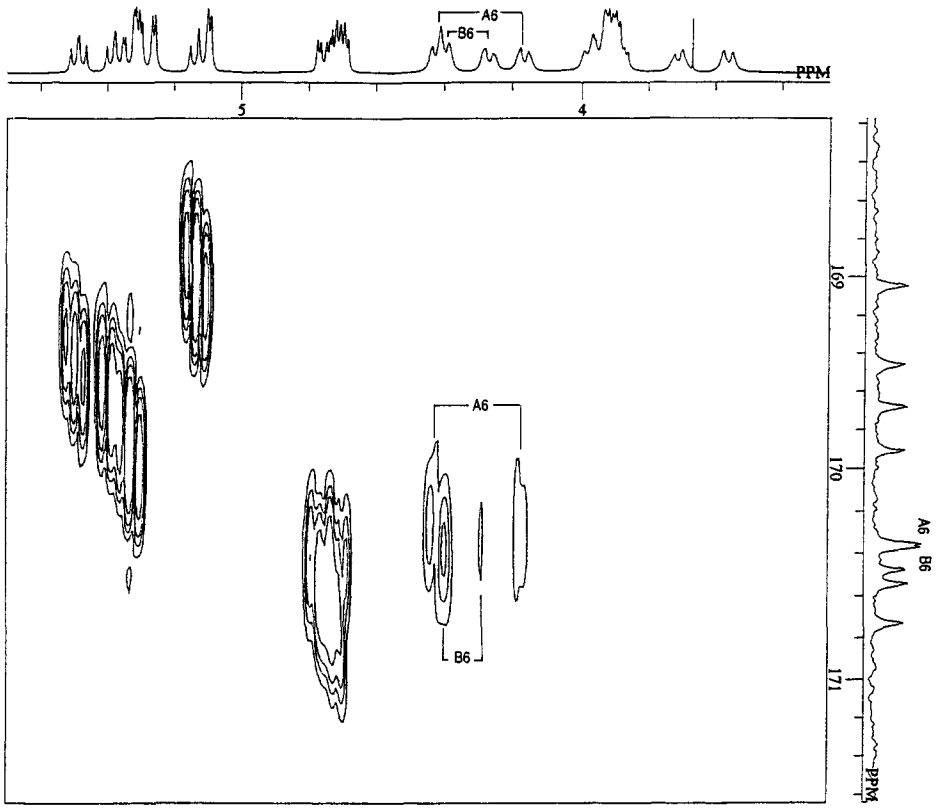

Fig. 5. The 400 MHz HMBC spectrum of pullulan peracetate in $CDCl_3$ at 40 °C, displaying connectivity between maltotriosyl protons and acetyl carbonyl carbons. For the glucosyl unit A, B and C; 4)-α-D-Glc^A-(1 → 4)-α-D-Glc^B-(1 → 6)-α-D-Glc^C-(1 → , see also the structural formula given in Fig. 1.

from specific protons in a glucose residue to acetyl or acyl carbonyl carbons can be detected more efficiently than by a normal-mode 2D long-range C–H COSY measurement. In the case of pullulan peracetate, however, the INAPT measurement failed to produce, for most protons irradiated by the INAPT long pulse, any detectable polarization-transfer peak, even with more than 50,000 transients.

An alternative 2D method for detecting a long-range heteronuclear correlation is an HMBC (heteronuclear multibond coherence) technique [18], which may be coupled with a field-gradient technique [20].

An HMBC measurement of pullulan peracetate showed a variety of long-range correlation peaks with high sensitivity. As shown in Fig. 3, the four-bond heteronuclear connectivity (*C*–O–CO–C–*H*) between acetyl protons and maltotriosyl carbons can be clearly observed, and all of the nine peaks in the acetyl proton signal can be directly correlated to the relevant carbon signal in the maltotriosyl unit. The assignment of the nine peaks in the acetyl proton signal is listed in Table 1 (third column).

The two-bond heteronuclear connectivity (*C*O–C–*H*) between acetyl protons and acetyl carbonyl carbons can also be detected, as shown in Fig. 4. Seven of the nine peaks in the acetyl carbonyl carbon signal are directly assignable from the observed correlation peaks, but the chemical shifts of the two C-6 peaks at A6 and B6 are so close to each other for correlation to the two resolved H-6 peaks.

The assignment of these two C-6 peaks can be achieved through observation of the three-bond heteronuclear connectivity (*H*–C–O–*C*O) between maltotriosyl protons and acetyl carbonyl carbons. In Fig. 5, the two-sets of correlation peaks between the relevant H-6 signals and the C-6 are observed, and remarkably the two acetyl carbonyl signals at the A6 and B6 positions can be distinguished, despite the minimal chemical-shift difference between them. Full assignment of the nine peaks in the acetyl carbonyl carbon signals is thus complete, and data are collected in Table 1 (last column).

In conclusion, the present paper demonstrates full assignment of the nine peaks in both the acetyl proton and acetyl carbonyl carbon signals in pullulan peracetate, by making use of 2D-NMR techniques, in particular of an HMBC technique. The chemical shift of both proton and carbon signals is confirmed to be

remarkably sensitive both to location within the glucose residues and to the linkage mode between them. Hence, the present results should provide a basis for further structural studies of not only pullulan derivatives, but also other polysaccharide derivatives of complex structures [21,22].

Acknowledgements

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References

[1] A. LeDuy, L. Choplin, J.E. Zajic, and J.H.T. Luong, *Encyclopedia of Polymer Science and Technology*, 2nd ed., Vol. 13, Wiley, New York, 1989, pp. 650–660.
[2] D. Bruneel and E. Schacht, *Polymer*, 34 (1993) 2628–2632.
[3] D. Bruneel and E. Schacht, *Polymer*, 34 (1993) 2633–2637.
[4] D. Bruneel and E. Schacht, *Polymer*, 35 (1994) 2656–2658.
[5] H.J. Jennings and I.C.P. Smith, *J. Am. Chem. Soc.*, 95 (1973) 606–608.
[6] A.J. Benesi and D.A. Brant, *Macromolecules*, 18 (1985) 1109–1116.
[7] P. Colson, H.J. Jennings, and I.C.P. Smith, *J. Am. Chem. Soc.*, 96 (1974) 8081–8087.
[8] C. Arnosti and D.J. Repeta, *Starch*, 47 (1995) 73–75.
[9] D. Bruneel, E. Schacht, and A. DeBruyn, *J. Carbohydr. Chem.*, 12 (1993) 769–778.
[10] Y. Tezuka, K. Imai, M. Oshima, and T. Chiba, *Macromolecules*, 20 (1987) 2413–2423.
[11] Y. Tezuka, K. Imai, M. Oshima, and T. Chiba, *Polymer*, 30 (1989) 2288–2291.
[12] Y. Tezuka, K. Imai, M. Oshima, and T. Chiba, *Carbohydr. Res.*, 196 (1990) 1–10.
[13] Y. Tezuka, K. Imai, M. Oshima, and T. Chiba, *Makromol. Chem.*, 191 (1990) 681–690.
[14] Y. Tezuka, K. Imai, M. Oshima, and T. Chiba, *Polym. J.*, 23 (1991) 189–193.
[15] Y. Tezuka, *Carbohydr. Res.*, 241 (1993) 285–290.
[16] Y. Tezuka and Y. Tsuchiya, *Carbohydr. Res.*, 273 (1995) 83–91.
[17] Y. Tezuka and Y. Tsuchiya, *Carbohydr. Res.*, 291 (1996) 99–108.
[18] M.F. Summers, L.G. Marzilli, and A. Bax, *J. Am. Chem. Soc.*, 108 (1986) 4285–4294.
[19] Y. Tezuka, *Biopolymers*, 34 (1994) 1477–1482.
[20] T.J. Norwood, *Chem. Soc. Rev.*, (1994) 59–66.
[21] J.M. van Hazendonk, E.J.M. Reinerink, P. de Waard, and E.G. van Dam, *Carbohydr. Res.*, 291 (1996) 141–154.
[22] B. Laignel, C. Bliard, G. Massiot, and J.M. Nuzzillard, *Carbohydr. Res.*, 298 (1997) 251–260.