Carbohydrate Research, 224 (1992) 277–283
Elsevier Science Publishers B.V., Amsterdam

Note

Preparation and n.m.r. assignments of cellulose mixed esters regioselectively substituted by acetyl and propanoyl groups

Tadahisa Iwata, Jun-Ichi Azuma*, Keizo Okamura,
Faculty of Agriculture, Kyoto University, Sakyo-ku, Kyoto 606-01 (Japan)

Mieko Muramoto, and
Fundamental Research Laboratory of Fiber and Fiber-Forming Polymers, Asahi Chemical Industry, Co. Ltd., 11-7, Hatchonawate, Takatsuki, Osaka 569 (Japan)

Byongwa Chun
W. R. Grace & Co. Japan Research Center, 100 Kanada, Atugi, Kanagawa 293 (Japan)

(Received May 9th, 1991; accepted in revised form July 10th, 1991)

Among cellulose triesters, only cellulose tripropanoate (CTP) has been shown to have threefold screw symmetry along the molecular axis. Other triesters — cellulose triacetate, cellulose tributanoate, and cellulose tripentanoate — have twofold screw symmetry along the molecular axis[1–3]. Questions arise. Why does only CTP possess the threefold screw symmetry? Which propanoyl groups play a decisive role in the conformation of the molecule, the primary substituent at C-6 or the two secondary substituents at C-2 and C-3? These are the motives of the present investigation.

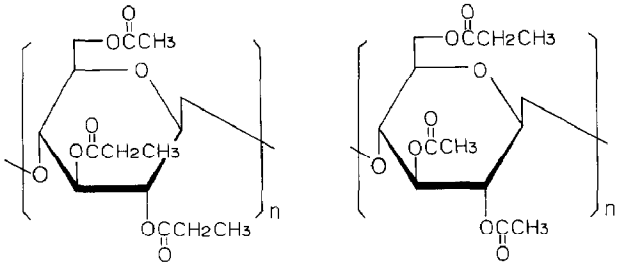
Recently developed nuclear magnetic resonance (n.m.r.) techniques are useful for the structural characterization of cellulose triesters[4–8]. We previously assigned all n.m.r. signals of CTP by a combination of 2D-n.m.r. and 1D-long-range selective proton decoupling (LSPD) techniques[9]. Although 2D-long-range heteronuclear shift-correlation spectroscopy (COLOC experiments[10,11]) should give the position of esterification, the assignment of both carbonyl carbons and acyl protons could not be achieved in a single experiment because of low sensitivity attributable to the combined effect of three- and two-bond couplings between these two atoms during refocusing period, and the short spin–spin relaxation-times of ring protons[7–9,12]. Recently, Frey *et al.*[13] and Bax *et al.*[14,15] presented a high-sensitivity 2D-heteronuclear multiple-bond connectivity (HMBC) technique designed to detect long-range couplings.

We report herein procedures for preparation of cellulose acetate dipropanoate (CADP, 6-*O*-acetyl-2,3-di-*O*-propanoyl-cellulose, **1**) and cellulose propanoate diacetate (CPDA, 2,3-di-*O*-acetyl-6-*O*-propanoyl-cellulose, **2**) and a full assigment of their n.m.r. signals by applying the HMBC technique together with the conventional 2D-

* To whom correspondence should be addressed.

0008-6215/92/$ 05.00

n.m.r. techniques. This work was to assure that we are dealing with the desired two cellulose mixed esters prior to undertaking detailed conformational analysis.



1 2

The ring ^{1}H- and ^{13}C-n.m.r. signals of CADP (**1**) were readily assigned by combination of the H,H-COSY and H,C-COSY experiments as listed in Table I. Similarly, the signals due to acyl methyl and methylene protons and carbons were identified, but no information about the sites of esterification was available as *J*-networks. For this purpose, it was necessary to detect three-bond couplings between ring protons and carbonyl carbons. This was achieved by the HMBC experiment. This

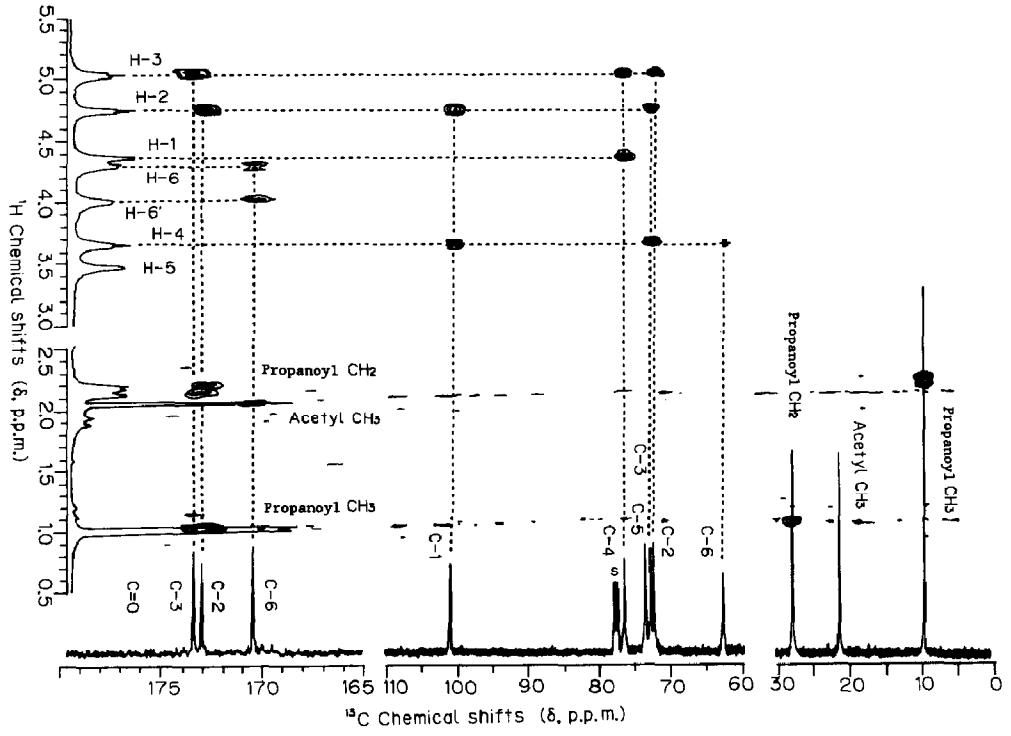


Fig. 1. ^{1}H-Detected ^{1}H–^{13}C multiple-bond correlation (HMBC) spectrum of cellulose acetate dipropanoate (CADP, 6-*O*-acetyl-2,3-di-*O*-propanoyl-cellulose, **1**). The symbol, s, denotes signals due to the solvent.

TABLE I

^{1}H- and ^{13}C-n.m.r. Data for 6-*O*-acetyl-2,3-di-*O*-propanoyl-cellulose (CADP, **1**)[a]

^{1}H-N.m.r. data (δ, p.p.m.)

Ring protons							*Acetyl protons*	*Propanoyl protons*			
							Methyl	*Methylene*		*Methyl*	
H-1	H-2	H-3	H-4	H-5	H-6	H-6′	6	2	3	2	3
4.41	4.82	5.12	3.73	3.54	4.37	4.07	2.15	2.27	2.22	1.08	1.06
(d)[b]	(t)	(t)	(t)	(m)	(m)	(m)	(s)	(q)	(q)	(t)	(t)
[7.7][c]	[8.4]	[8.8]	[8.6]							[7.6]	[7.6]

^{13}C-N.m.r. data (δ, p.p.m.)

Ring carbons						*Acetyl carbons*	*Propanoyl carbons*			
						Methyl	*Methylene*		*Methyl*	
C-1	C-2	C-3	C-4	C-5	C-6	6	2	3	2	3
100.63	72.02	72.49	76.10	73.17	62.40	21.09	27.56	27.56	9.23	9.23
							Carbonyl carbons			
							2	3	6	
							173.04	173.46	170.51	

[a] In $CDCl_3$ at 25°. [b] Multiplicities of signals. [c] ^{1}H–^{1}H coupling constant (Hz).

experiment, first developed to characterize short DNA duplexes[13] and coenzyme B_{12} (refs. 14, 15), has been extended to various compounds, including *Haemophilus influenzae* type *a* capsular polysaccharide[16], but has not been applied to cellulose derivatives.

The HMBC spectrum of CADP (1) is shown in Fig. 1. Although H-5 and H-6 signals did not show a clear correlation peak, as noticed in the results of Byrd *et al.*[16], the signals for the other ring protons could be immediately assigned from the HMBC connectivity map.

Both the H-2 and H-3 signals had two intense HMBC correlation peaks with C-1 and C-3, and C-2 and C-4, respectively, based on two-bond couplings. The glycosidic linkage site, assigned as the H-1 signal, gave an HMBC peak with C-4 of the neighboring glucose residue, based on three-bond coupling across an *O*-linked glycosidic linkage. In turn, the H-4 signal gave an HMBC correlation peak with C-1 of the neighboring glucose residue in addition to correlation with C-3 and C-6.

The locations of acyl groups were unambiguously determined by the HMBC experiment. The acyl carbonyl carbon signals at δ 173.46, 173.04, and 170.51 gave HMBC correlation peaks with the signals of protons at the sites of esterification, H-3, H-2, and H-6, respectively. The methyl and methylene protons of the same acyl groups also correlated the corresponding carbonyl carbons. In addition, the HMBC experiment showed correlation peaks between propanoyl methyl and methylene groups. The

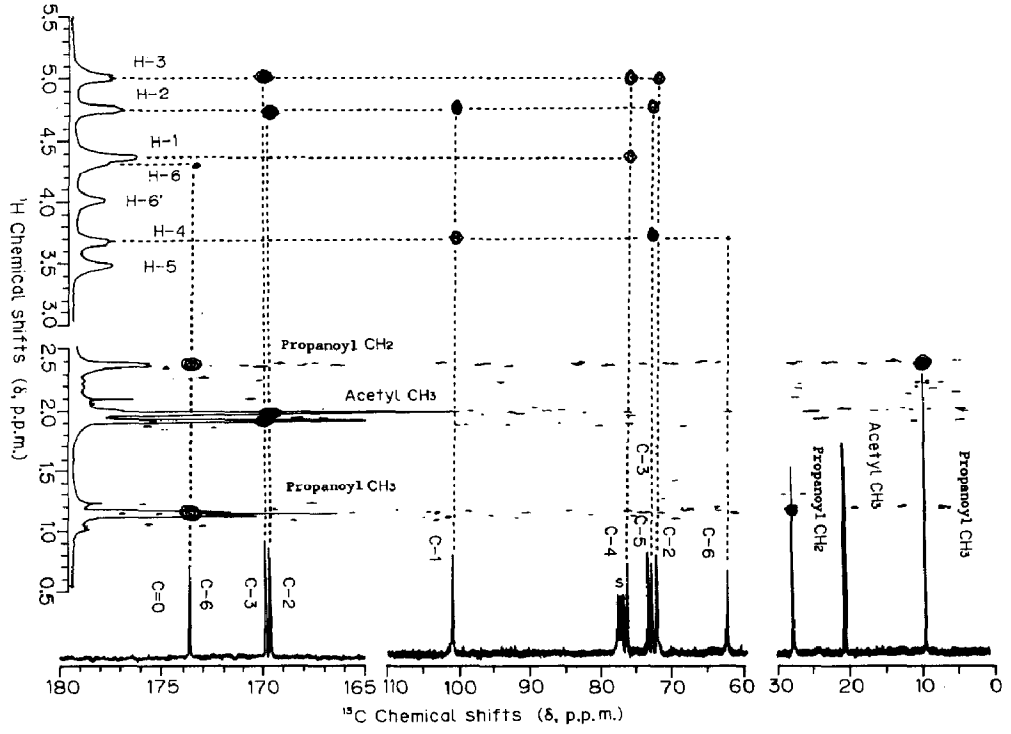


Fig. 2. ^{1}H-Detected ^{1}H–^{13}C multiple-bond correlation (HMBC) spectrum of cellulose diacetate propanoate (CPDA, 6-*O*-acetyl-2,3-di-*O*-propanoyl-cellulose, **2**). The symbol, s, denotes signals due to the solvent.

TABLE II

^{1}H- and ^{13}C-n.m.r. Data for 2,3-di-*O*-acetyl-6-*O*-propanoyl-cellulose (CPDA, **2**)[a]

^{1}H-N.m.r. data (δ, p.p.m.)

Ring protons							*Acetyl protons*		*Propanoyl protons*	
							Methyl		*Methylene*	*Methyl*
H-1	H-2	H-3	H-4	H-5	H-6	H-6′	2	3	6	6
4.39	4.78	5.05	3.70	3.54	4.36	4.05	2.01	1.93	2.40	1.18
(d)[b]	(t)	(t)	(t)	(m)	(m)	(m)	(s)	(s)	(q)	(t)
[7.8][c]	[8.3]	[8.8]	[8.8]							[7.6]

^{13}C-N.m.r. data (δ, p.p.m.)

Ring carbons						*Acetyl carbons*		*Propanoyl carbons*	
						Methyl		*Methylene*	*Methyl*
C-1	C-2	C-3	C-4	C-5	C-6	2	3	6	6
100.79	72.02	72.72	76.34	73.14	62.21	20.75	20.67	27.58	9.28

Carbonyl carbons		
2	3	6
169.48	169.93	173.84

[a] In $CDCl_3$ at 25°. [b] Multiplicities of signals. [c] ^{1}H–^{1}H coupling constant (Hz).

assignments achieved with the HMBC experiment are given in Table I, which provides a complete n.m.r. assignment of CADP (**1**). The n.m.r. assignments of the other cellulose mixed ester, CPDA (**2**), in which the acetyl and propanoyl groups are interchanged, were straightforward and the results are shown in Fig. 2 and Table II.

In summary, the HMBC experiment has been found to provide a rapid and a reliable method for unambiguous establishment of the substitution sites of cellulose esters.

EXPERIMENTAL

Materials. — Cellulose derivatives were prepared from low-molecular-weight cellulose having a weight-average degree of polymerization (dp_w) of 57. The cellulose (1 g) was dissolved in 60 mL of *N,N*-dimethylacetamide containing 7.5% (w/v) of LiCl[17], and tritylated selectively at O-6 by 4.3 g of chlorotriphenylmethane (2.5 mol per mole of OH) in 23 mL of pyridine for 48 h at 100°. The degree of substitution (d.s.) of the 6-*O*-tritylcellulose precipitated by MeOH was determined[18] to be 0.92. The 6-*O*-tritylcellulose (1 g) was dissolved in 18 mL of pyridine and propanoylated with 16.1 mL of propanoic anhydride (25 mol per mole of OH) for 48 h at 90°, and then detritylated with 2 mL of 25% HBr in HOAc in 34 mL of a mixture of HOAc and $CHCl_3$ (2:15, v/v). After vigorous stirring for 5 min at room temperature, the mixture was poured into 15 vol. of 50% aqueous MeOH and made neutral immediately by adding solid $NaHCO_3$. The precipitated material was recovered by centrifugation, washed with MeOH, and dried *in vacuo* to give 2,3-di-*O*-propanoyl-cellulose which was then acetylated with a mixture of pyridine and Ac_2O as already described to give cellulose acetate dipropanoate (CADP, 6-*O*-acetyl-2,3-di-*O*-propanoyl-cellulose, **1**) in a yield of 60%.

Similarly, cellulose propanoate diacetate (CPDA, 2,3-di-*O*-acetyl-6-*O*-propanoyl-cellulose, **2**) was prepared in 61% yield by interchanging Ac_2O and propanoic anhydride in the preceding procedure.

The purities of CADP (**1**) and CPDA (**2**) were estimated to be 91 and 92%, respectively, by ^{1}H-n.m.r. spectroscopic measurements. The dp_w values of CPDA and CADP were estimated to be 46.4 and 32.0, respectively, by gel-permeation chromatography on a column of Shodex A-80M (8.0 mm x 50 cm) eluted with $CHCl_3$ and calibrated against CTP.

Measurements. — All n.m.r. experiments were performed with a GE NMR Instruments Omega 500 spectrometer at 500.14 MHz for ^{1}H and 125.77 MHz for ^{13}C. The spectra were recorded using 50 mg of sample in 0.6 mL of $CDCl_3$ at 25°. Chemical shifts in p.p.m. were downfield from the internal Me_4Si (1%, v/v). HMBC, H,H-COSY, and H,C-COSY experiments were carried out with the pulse sequence and programs provided by GE. HMBC spectra were the results from a 1024 x 256 data-matrix size with 128 scans and a delay time between scans of 1.0 s. The durations Δ_1 and Δ_2 were 3.5 and 67.9 ms, respectively.

REFERENCES

1 P. Zugenmaier, *J. Appl. Polym. Sci.: Appl. Polym. Symp.*, 37 (1983) 223–238.
2 Y. Shuto, K. Okamura, J. Azuma, F. Tanaka, and H. Chanzy, in C. Schuerch, Ed., *Cellulose and Wood: Chemistry and Technology*, Wiley, New York, 1989, pp. 207–220.
3 Y. Shuto, K. Okamura, J. Azuma, F. Tanaka, and H. Chanzy, in J. F. Kennedy, G. O. Phillips, and P. A. Williams, Eds., *Cellulose: Structural and Functional Aspects*, Ellis Horwood Ltd., Chichester, England, 1989, pp. 283–288.
4 K. Kowsaka, K. Okajima, and K. Kamide, *Polym. J.*, 18 (1986) 843–849.
5 K. Kowsaka, K. Okajima, and K. Kamide, *Polym. J.*, 20 (1988) 1091–1099.
6 C. M. Buchanan, J. A. Hyatt, and D. W. Lowman, *Macromolecules*, 20 (1987) 2750–2754.
7 C. M. Buchanan, J. A. Hyatt, and D. W. Lowman, *Carbohydr. Res.*, 177 (1988) 228–234.
8 P. Dais and A. S. Perlin, *Carbohydr. Res.*, 181 (1988) 233–235.
9 Y. Shuto, M. Murayama, J. Azuma, and K. Okamura, *Bull. Inst. Chem., Kyoto Univ.*, 66 (1988) 128–135.
10 H. Kessler, C. Griesinger, J. Zarbock, and H. R. Loosli, *J. Mag. Reson.*, 57 (1984) 331–336.
11 H. Kessler, W. Bermel, and C. Griesinger, *J. Am. Chem. Soc.*, 107 (1985) 1083–1084.
12 S. Uhrínová, E. Petráková, J. Ruppeldt, and D. Uhrín, *Magn. Reson. Chem.*, 28 (1990) 979–987.
13 M. H. Frey, W. Leupin, O. W. Sørensen, W. A. Denny, R. R. Ernst, and K. Wüthrich, *Biopolymers*, 24 (1985) 2371–2380.
14 A. Bax and M. F. Summers, *J. Am. Chem. Soc.*, 108 (1986) 2093–2094.
15 M. F. Summers, L. G. Marzilli, and A. Bax, *J. Am. Chem. Soc.*, 108 (1986) 4285–4294.
16 R. A. Byrd, W. Egan, M. F. Summers, and A. Bax, *Carbohydr. Res.*, 166 (1987) 47–58.
17 C. L. McCormick, P. A. Callais, and B. H. Hurchinson, Jr., *Macromolecules*, 18 (1985) 2394–2401.
18 J. W. Green, *Methods Carbohydr. Chem.*, 3 (1963) 327–331.