

## NMR Assignments of Propionyl Groups in Cellulose Tripropionate

Yuichiro SHUTO\*, Masahiko MURAYAMA\*\*, Jun-ichi AZUMA\*,  
and Keizo OKAMURA\*

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All the NMR signals of cellulose tripropionate were completely assigned by a combined use of the techniques of  $^1\text{H}$ - $^1\text{H}$  homonuclear shift-correlation spectroscopy (H, H-COSY),  $^1\text{H}$ - $^{13}\text{C}$  heteronuclear shift-correlation spectroscopy (H, C-COSY),  $^1\text{H}$ - $^{13}\text{C}$  shift-correlation spectroscopy *via* long-range couplings (H, C-COLOC), and long-range selective proton decoupling (LSPD) with special emphasis on the signal assignments for propionyl groups.

**KEY WORDS:** Cellulose tripropionate/ NMR assignments/ 2D-NMR/ Propionyl/ Propionyl carbonyl/ Propionyl methyl/ Propionyl methylene/

### INTRODUCTION

A series of aliphatic cellulose esters have been systematically studied in order to investigate the conformation-function relationships in cellulose derivatives.<sup>1-3)</sup> It has been shown that, based on X-ray diffraction data, the conformation of cellulose tripropionate (CTP) is unique showing threefold helical symmetry which has not been observed in the other cellulose ester homologues.<sup>4,5)</sup> We prepared ribbonlike lamellar single crystals of CTP, and proposed new orthogonal lattice parameters and the two dimensional space group based on the electron diffraction spots.<sup>6)</sup> Combining the electron diffraction data with X-ray diffraction data of the oriented CTP film, we further reported on the conformation of single CTP chain.<sup>7)</sup> Prior to a detailed conformational analysis, it would be legitimate to report on the full chemical characterization of CTP.

Recent progress in one- and two-dimensional NMR spectroscopy has enabled one to do the complete signal assignment of  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR spectra of cellulose triacetate (CTA).<sup>8-10)</sup> In the case of CTP, although its NMR signals in the ring protons and carbons have been assigned by Buchanan *et al.*,<sup>9)</sup> the signals due to propionyl groups have never been reported.

In this study, we present unambiguous NMR assignments of the signals of propionyl groups of CTP by a combined use of various two-dimensional and long-range selective proton decoupling (LSPD) techniques.

\* 首藤勇一郎, 東 順一, 岡村圭造: Department of Wood Science and Technology, Faculty of Agriculture, Kyoto University, Sakyo-ku, Kyoto 606

\*\* 村山雅彦: Ashigara Research Laboratories, Fuji Photo Film Co., Ltd., Minami-ashigara, Kanagawa 250-01

## EXPERIMENTAL

*NMR spectroscopic measurement.* All the NMR experiments were carried out with a Bruker AM-300 spectrometer ( $^1\text{H}$  at 300.1 MHz and  $^{13}\text{C}$  at 75.5 MHz) using 5 mm-diameter sample tubes at 25°C in  $\text{CDCl}_3$ . Chemical shifts were relative to the internal  $\text{Me}_4\text{Si}$ . The  $^1\text{H}$ - $^1\text{H}$  homonuclear shift-correlation spectroscopy (H, H-COSY) and  $^1\text{H}$ - $^{13}\text{C}$  heteronuclear shift-correlation spectroscopy (H, C-COSY) experiments were carried out using the pulse sequences of Bax and Freeman<sup>11)</sup> and Bax,<sup>12)</sup> respectively. The  $^1\text{H}$ - $^{13}\text{C}$  shift-correlation spectroscopy *via* long-range couplings (H, C-COLOC)<sup>13)</sup> was measured with the microprograms (COLOC. AU) supplied by Bruker using delays of relaxation (1 sec), evolution ( $\Delta_1$ , 90  $\mu\text{sec}$ ), and refocusing ( $\Delta_2$ , 40  $\mu\text{sec}$ ). The long-range selective proton decoupling (LSPD)<sup>14)</sup> experiments were performed by keeping irradiation power low enough to decouple long-range C-H couplings of few Hz.

*Preparation of cellulose tripropionate.* The propionylation of cellulose was carried out by using trifluoroacetic anhydride as an impelling agent of esterification.<sup>15)</sup> Two grams of dried Avicel SF cellulose powder (Asahi Kasei Kogyo, Co., Ltd.) was poured into a mixture 100 ml of propionic acid and trifluoroacetic anhydride (0.57:0.44, mol/mol) which was previously heated for 20 min at 50°C to make the mixed solution compatible, and the reaction mixture was vigorously stirred at the same temperature for 12 h. The resulting slightly brownish clear solution was cooled to room temperature, poured into 5 vol. of methanol and kept over-night at 5°C. The white precipitate formed was recovered by filtration through Whatman No. 2 filter paper and washed thoroughly with methanol. The crude CTP was then solubilized by addition of minimum amount of chloroform and subsequently poured into 5 vol. of methanol. This solubilization and precipitation procedure was repeated once more to give pure CTP in 70.5% yield. IR spectrum of CTP (Shimadzu FTIR-4000) showed a strong absorption at  $1,750\text{ cm}^{-1}$  due to carbonyl groups with no absorption for free hydroxyl groups. The degree of substitution of CTP was estimated to be 3.0 from its  $^1\text{H}$  NMR spectrum.

*Molecular weight determination of cellulose tripropionate.* Molecular weight of CTP was determined by two different methods, gel permeation chromatography (GPC) and sedimentation equilibrium.<sup>16)</sup> GPC was conducted on a Waters Assoc. (Milford, Madison, U.S.A.) instrument with a model 6000A pump, model U6K sample injector, model R401 differential refractometer, and model 730 data module. The column used was a Shodex A-80M column (8 mm $\times$ 50 cm) and chloroform was used as an eluting solvent. The flow rate and the pressure of the column were 1.0 ml/min and 3.1 MPa, respectively. The weight average molecular weight of CTP was estimated to be 66,000 by using polystyrenes (Shimadzu Standard Polystyrenes) as calibration standards. The sedimentation equilibrium was carried out in acetone<sup>17)</sup> at 16,200 rpm and 25°C using a Beckman Model E ultracentrifuge equipped with a Rayleigh interferometric optical system. The molecular weight was calculated to be 34,000 by using values of solvent density (0.792 g/ml) and partial specific volume of CTP (0.818 ml/g).

## RESULTS and DISCUSSION

*Ring proton and carbon signals.* Previously, Buchanan *et al.*<sup>9)</sup> tried to assign the ring proton and carbon signals of CTP. In this study, we at first evaluated their results by means of the  $^1\text{H}$ ,  $^1\text{H}$ -COSY and  $^1\text{H}$ ,  $^{13}\text{C}$ -COSY experiments. Based on the  $^1\text{H}$ - $^{13}\text{C}$  shift-correlation map shown in Fig. 1 with each peak denoted with its assignment in the projection spectra together with  $^1\text{H}$ - $^1\text{H}$  shift-correlation map (data not shown), all the signals in the ring proton and carbon region could be assigned and the results are summarized in Table 1. The present assignments verified the previous results of Buchanan *et al.*,<sup>9)</sup> and further included a signal of H-6s.

*Carbonyl carbon signals.* Assignment of the carbonyl carbon signals in aliphatic acid cellulose esters may yield valuable information for determination of degree and positional distribution of substitution.<sup>8,18,19)</sup> Because of the absence of direct chemical bonding between glucose residues and propionyl groups through oxygen atom, it is necessary to use NMR techniques specifically designed to detect  $^1\text{H}$ - $^{13}\text{C}$  spin-spin couplings through two and three bonds.<sup>10,13,14,20,21)</sup> In the case of CTA, all carbonyl carbon signals were unambiguously determined by using LSPD<sup>8)</sup> and INAPT (insensitive nuclei assigned by polarization transfer)<sup>10)</sup> techniques. In this study, we applied the LSPD technique to assign the propionyl carbonyl carbon signals of CTP. Figure 2a shows the proton decoupled  $^{13}\text{C}$ -NMR spectrum for the carbonyl carbon region of CTP, indicating the presence of three signals at 173.6, 173.2, and 172.7 p.p.m. Figure 2b shows the gated decoupled spectrum obtained without nuclear Overhauser effect (n.O.e.) in which the signals in the spectrum 2a became broad and split in agreement with the results obtained in CTA.<sup>8)</sup> Hereupon, the specific ring protons of H-2, 3 and 6 were sequentially decoupled and the LSPD spectra are recorded as shown in Fig. 2c-2e.

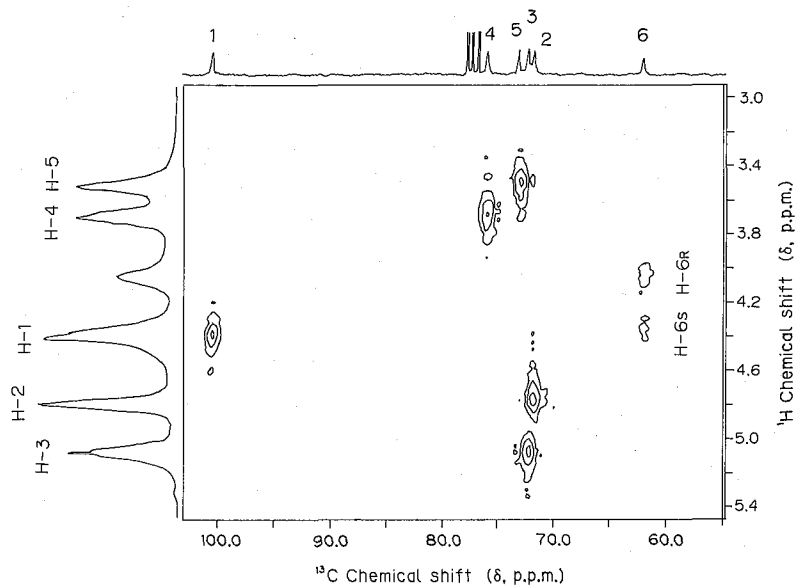


Fig. 1.  $^1\text{H}$ ,  $^{13}\text{C}$ -COSY spectrum of cellulose tripropionate in the ring proton and carbon region.

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Table 1.  $^1\text{H}$  and  $^{13}\text{C}$  chemical shifts and  $^1\text{H}$ - $^1\text{H}$  coupling constants for cellulose tripropionate<sup>a)</sup>

| <sup>1</sup> H-NMR data (δ, p.p.m.) |       |       |       |      |      |      |                   |       |       |        |      |      |
|-------------------------------------|-------|-------|-------|------|------|------|-------------------|-------|-------|--------|------|------|
| Ring protons                        |       |       |       |      |      |      | Propionyl protons |       |       |        |      |      |
|                                     |       |       |       |      |      |      | Methylene         |       |       | Methyl |      |      |
| H-1                                 | H-2   | H-3   | H-4   | H-5  | H-6s | H-6R | 2                 | 3     | 6     | 2      | 3    | 6    |
| 4.39                                | 4.79  | 5.08  | 3.69  | 3.52 | 4.37 | 4.05 | 2.25              | 2.18  | 2.39  | 1.06   | 1.03 | 1.18 |
| (d) <sup>b)</sup>                   | (t)   | (t)   | (t)   | (m)  | (m)  | (m)  | (q)               | (q)   | (q)   | (t)    | (t)  | (t)  |
| [7.9] <sup>c)</sup>                 | [8.5] | [9.0] | [9.1] |      |      |      | [7.4]             | [7.4] | [7.4] |        |      |      |

| <sup>13</sup> C-NMR data (δ, p.p.m.) |       |       |       |       |       |                   |        |        |        |      |      |  |
|--------------------------------------|-------|-------|-------|-------|-------|-------------------|--------|--------|--------|------|------|--|
| Ring carbons                         |       |       |       |       |       | Propionyl carbons |        |        |        |      |      |  |
|                                      |       |       |       |       |       | Methylene         |        |        | Methyl |      |      |  |
| C-1                                  | C-2   | C-3   | C-4   | C-5   | C-6   | 2                 | 3      | 6      | 2      | 3    | 6    |  |
| 100.37                               | 71.81 | 72.25 | 75.90 | 73.08 | 62.04 | 27.28             | 27.17  | 27.38  | 8.93   | 8.90 | 9.09 |  |
|                                      |       |       |       |       |       | Carbonyl          |        |        |        |      |      |  |
|                                      |       |       |       |       |       |                   |        |        |        |      |      |  |
|                                      |       |       |       |       |       | 2                 | 3      | 6      |        |      |      |  |
|                                      |       |       |       |       |       | 172.71            | 173.15 | 173.62 |        |      |      |  |

<sup>a)</sup> In  $\text{CDCl}_3$  at  $25^\circ\text{C}$ .

<sup>b)</sup> Multiplicities of signals.

<sup>c)</sup>  $^1\text{H}$ - $^1\text{H}$  coupling constant (Hz).

Irradiation at the frequency of H-2 (4.79 p.p.m.) changed the broad signal at 172.7 p.p.m. into a doublet-like one. Similarly, the shapes of the signals at 173.2 p.p.m. and 173.6 p.p.m. were changed by irradiation at the frequencies of H-3 (5.08 p.p.m.) and H-6R (4.05 p.p.m.), respectively. All the propionyl carbonyl signals could thus be unequivocally assigned as listed in Table 1.

Recently, the H, C-COLOC technique has been developed to detect specifically two- and three-bond  $^1\text{H}$ - $^{13}\text{C}$  spin-spin couplings<sup>13)</sup>, and applied to mono- and oligo-saccharides.<sup>22)</sup> Although we applied this technique to CTP, no clear connectivity between carbonyl carbons and ring protons could be observed under the present experimental condition as suggested by Buchanan *et al.*<sup>10)</sup>

*Propionyl methyl and methylene signals.* Since the propionyl methyl and methylene protons were separated from carbonyl carbons by two- or three-bonds, the same NMR techniques that were used to assign carbonyl carbon signals were expected to be applicable. We now applied the H, C-COLOC technique for this purpose. Figure 3 shows a H, C-COLOC spectrum of CTP in the carbonyl carbon and methyl proton region (three-bond couplings) with each peak denoted with its assignment in the projection spectra. Three sets of triplet signals at 1.18, 1.06, and 1.03 p.p.m. could be assigned to propionyl methyl protons substituted at O-6, O-2, and O-3, respectively (Table 1). The assignment of the propionyl methyl protons were confirmed by the LSPD technique, *e.g.*, by irradiating of the protons at 1.18 p.p.m. the carbonyl carbon

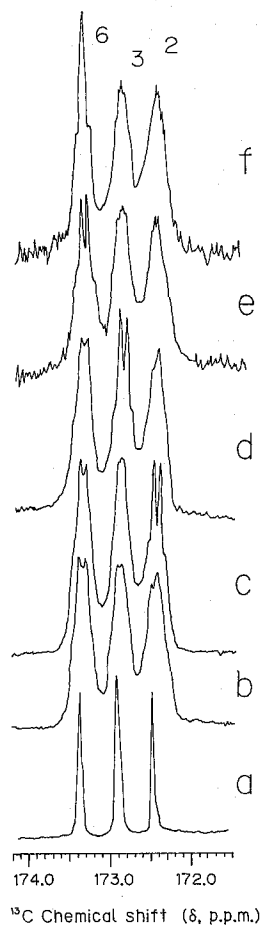


Fig. 2. LSPD spectra of cellulose tripropionate in the carbonyl carbon region with proton decoupled and gated decoupled spectra. (a) Proton decoupled; (b) Gated decoupled; (c) Selectively decoupled at H-2 (4.79 p.p.m.); (d) Selectively decoupled at H-3 (5.08 p.p.m.); (e) Selectively decoupled at H-6<sub>R</sub> (4.05 p.p.m.); (f) Selectively decoupled at methyl protons (1.18 p.p.m.).

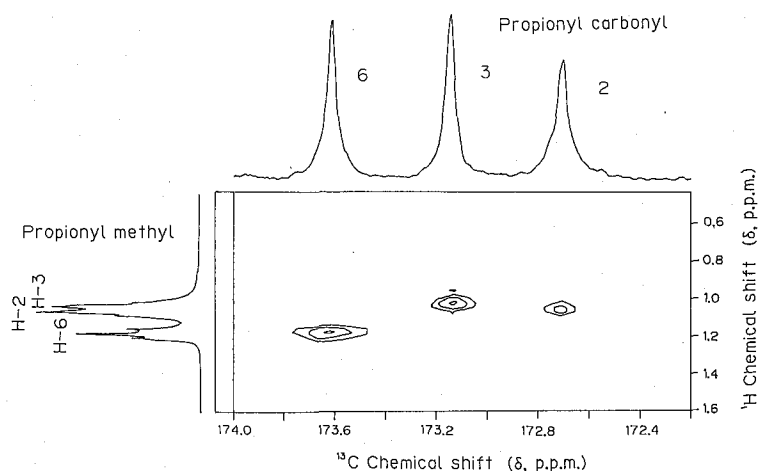


Fig. 3. COLOC spectrum of cellulose tripropionate in the methyl proton and carbonyl carbon region.

signal at 173.6 p.p.m. due to propionyl group substituted at O-6 became sharp as shown in Fig. 2f.

In the same  $^1\text{H}$ , C-COLOC spectrum, only one cross peak, instead of three, was also observed between carbonyl carbon at 173.6 p.p.m. and methylene protons at 2.39 p.p.m. (two-bond couplings).

In addition, it has been found that the signals of methylene carbons could be completely assigned from the cross peaks due to two-bond couplings with methyl protons in the same COLOC spectrum. The results are shown in Table 1 and Fig. 4 with each peak denoted with its assignment in the projection spectra. The connectivity between methyl carbons and methylene protons (two-bond couplings) were also observed in the COLOC spectrum. However, because of overlap of the two methyl carbons at higher field, only one cross peak at 9.09 p.p.m. could be assigned to methyl carbon of the propionyl group substituted at O-6.

All remaining signals of propionyl methyl carbons and methylene protons could be assigned unequivocally in the  $^1\text{H}$ , C-COSY spectrum. The representative results of  $^1\text{H}$ , C-COSY spectra are shown in Figs. 5 and 6 with each peak denoted with its assignment in the projection spectra. These assignments could also be alternatively carried out by combined use of the  $^1\text{H}$ , H-COSY and  $^1\text{H}$ , C-COSY techniques and completely confirmed the results presented above.

We have demonstrated that the techniques of LSPD and COLOC are most appropriate for unambiguous determination of esterified position of CTP. These

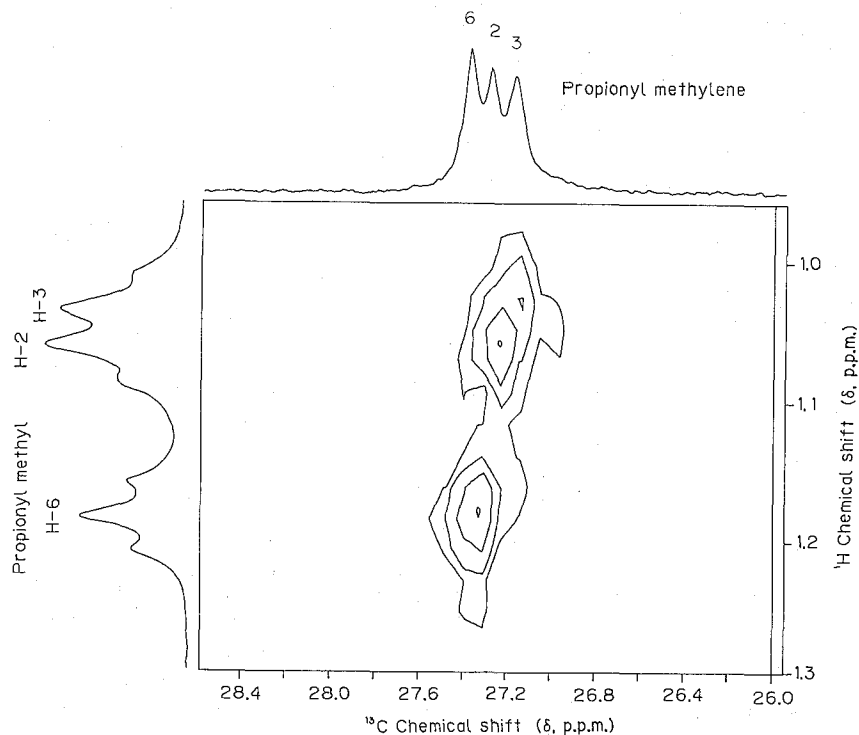


Fig. 4. COLOC spectrum of cellulose tripropionate in the methyl proton and methylene carbon region.

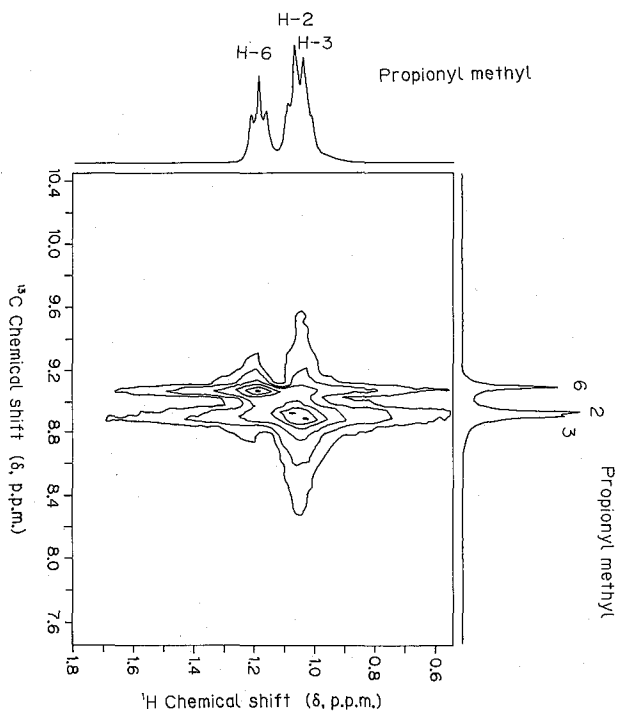


Fig. 5. H, C-COSY spectrum of cellulose tripropionate in the methyl proton and carbon region.

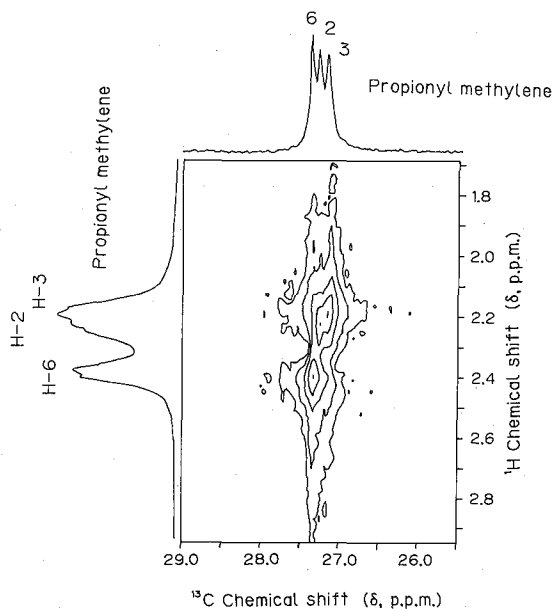


Fig. 6. H, C-COSY spectrum of cellulose tripropionate in the methylene proton and carbon region.

techniques might be applicable to the higher ester homologous series of aliphatic acid cellulose esters.

#### NOTE ADDED IN PROOF

Two papers have just been reported to confirm the signal assignments of acetyl carbonyl carbon atoms of CTA by using long-range H, C-COSY.<sup>23, 24)</sup>

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