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Studies on Chitin. VI. Preparation and Properties of Alkyl-Chitin Fibers

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ABSTRACT: Alkali-chitin was prepared by a simple procedure which included a freezing process in a sodium hydroxide-sodium dodecylsulfate system. Its alkylations were achieved with various alkyl halides of different chain lengths and bulkiness. The affinity toward water or formic acid was remarkably enhanced by the alkylations in spite of the low degree of substitution. The increase in hydrophilicity was considered to be result from the partial destruction of crystalline structure in the chitin molecule. The alkyl-chitin fibers were successfully prepared by spinning the solution of alkyl-chitin in formic acid-dichloroacetic acid mixture into ethyl acetate. The extent of swelling with water was found to correspond both to the chain length and the bulkiness of the alkyl group. The hydroxyl group at the C_6 position is considered to be substituted prior to that of C_3 position, according to a ^{13}C NMR study. ^1H NMR and elemental analysis proved useful in estimting the degree of alkylation even in the case of low degree of substitution.

KEY WORDS Chitin / Alkyl-Chitin / Freezing Process / Alkylation / Alkyl-Chitin Fiber / ^1H NMR / ^{13}C NMR / Elemental Analysis /

Chitin, a natural abundant polysaccharide, is known to be sparingly soluble in general organic solvents owing to its strong crystalline structure through hydrogen bonds. Successful regeneration of chitin as a fiber or film was carried out by methods in which chitin was dissolved in formic acid directly.[1] The preparation of acetyl-chitins and their fibers was also carried out successfully.[2,3] The acylation reactions of chitin with longer or more bulky groups were found to improve its solubility properties in organic solvents.[4,5]

In the present study, the partial destruction of the crystalline structure in chitin molecule was carried out so as to increase its affinity toward water by alkylation, using various groups of different chain lengths and bulkiness. The enhancement of affinity toward water or other solvents enables chitin to take on a greater number of uses. The bulkiness and chain length of the attached alkyl-groups were found to be major factors in increasing the hydrophilicity of chitin derivatives even in the case of low degree of substitution. The degree of alkylation estimated by ^{13}C or ^1H NMR was roughly comparable with that by elemental analysis. The initial alkylation site on the *N*-acetylglucosamine residue was suggested from ^{13}C NMR study to be the hydroxyl group at C_6 position.

EXPERIMENTAL

Chitin

Chitin, prepared from Queen Crab shell, was kindly supplied from Kyowa Yushi Co. Ltd. and it was powdered to a 45—60 mesh before use.

Reagents

Alkyl halides (methyl iodide, ethyl bromide, *n*-butyl bromide, *i*-butyl bromide, *t*-butyl bromide, *n*-amyl bromide, *i*-amyl bromide, and *t*-amyl bromide) were purchased from Wako Pure Chemical Industries Ltd. and used without further purification.

Alkali-Chitin

10 g of chitin powder was added to 40 ml of a 40% sodium hydroxide solution containing 0.2% sodium

dodecylsulfate (SDS) at 4°C and the mixture was made to stand for 1 h at 4°C until the chitin became swollen. The chitin sulurry was kept at −20°C overnight.

Alkylation of Chitin

The frozen alkali-chitin was suspended directly in alkyl halides (10 equivalent moles of alkyl halide for one *N*-acetylglucosamine residue) and the reaction was carried out at 12—14°C for 24 h with occasional mechanical stirring. The reaction mixture was neutralized with diluted acetic acid and the precipitate was collected by filtration. The product was dried in air after successive washing with ethanol, water, ethanol, and finally with acetone.

Estimation for the Degree of Alkylation

The degree of alkylation was estimated primarily from elemental analysis using a Yanagimoto CHN Corder MT-2, going on the assumption that a half mole of water was included per each *N*-acetylglucosamine residue.[2] As there was fairly good agreement between the theoretical and analytical values of elemental analysis as shown in Table I, chitin was not considered to be deacetylated during the preparation. An ^{1}H NMR measurement was also made for estimating the degree of alkylation, using a JEOL FX-400 (400 MHz) spectrometer in deuterated formic acid (DCOOD) at a polymer concentration of 1.8% and 45°C. The chemical shift was measured relative to that of HDO. ^{13}C NMR measurements were made with a JEOL FX-60Q (15 MHz) to estimate the initial alkylation site on the *N*-acetylglucosamine residue using 1.2 ethylated chitin in DCOOD at 45°C. The chemical shift was measured relative to the center peak of DCOOD. The degree of alkylation at C_6 position was calculated by a comparison of the peak areas between the alkylated and unreacted sites using the gated decoupling method. 1.2 ethylated chitin was prepared only for NMR analysis by reacting alkali-chitin with diethyl sulfate at 12—14°C for 24 h.

Preparation of Alkyl-Chitin Fibers

The fibers of alkyl-chitins were prepared by spinning the solution of alkyl-chitin in formic acid-dichloroacetic acid mixture into ethyl acetate, as was done in the preparation of chitin[1] or acetylchitin fibers[3] except there was no stretching bath. In contrast to the treatment of chitin fibers, the alkyl-chitin fibers were stretched in air immediately after the coagulation, since they quickly swell with water and become fragile. The stretched fibers were immersed in ethanol containing NaOH so as to remove any dichloroacetic or formic acid. The fibers were washed with ethanol successively and then dried in air.

Properties of Alkyl-chitin Fibers

The tensile strength, elongation and Young's modulus of alkyl-chitin fibers were measured by the method reported previously.[1]

Degree of Swelling of Fiber

The degree of swelling of alkyl-chitin fiber was estimated both by the microscopic observation and the titration of water with automatic Kirl–Fischer titration apparatus of Hirama Aquacounter AQ-3. In the microscopic observation, pictures were taken before and after contact with water, and the diameter of the dry and swollen fibers was measured from the pictures. In order to estimate the amount of absorbed water, fiber bandles were immersed in water at 25°C for 3 min, and the swollen fibers were subjected to an Aquacounter at 20°C following the removal of water from the surface of fibers with filter paper.

Heat Transition of Alkyl-Chitin

The energies of heat transition at 151—226°C were measured by differential scanning calorimetric analysis using differential scanning calorimeter Rigaku 8001-SL/C by applying a 45—60 mesh of alkyl-chitin powder against aluminum oxide. The energies of heat transition were estimated by making reference to that of freshly prepared urea (mp 130°C) at a heating rate of 5°C/min in sealed aluminum cell.

RESULTS AND DISCUSSION

Preparation of Alkali-Chitin

Sodium dodecylsulfate (SDS) and the freezing process were necessary for the preparation of water-soluble alkali-chitin with 40% sodium hydroxide solution. Alkali-chitin prepared without SDS or freezing process never disperses well in ice water. The penetration of the sodium hydroxide solution into the crystalline structure of chitin is probably promoted by a detergent such as SDS, and the

freezing process is considered to work for the destruction of the crystalline structure.

The deacetylation from chitin molecule is not considered to occur during the preparation of alkali-chitin, since all procedures were carried out below 5°C. This was confirmed by Noguchi *et al.*[6] The extent of deacetylation from chitin molecule was also assumed to be less than 5%, according to potentiometric titration of water-soluble carboxymethyl-chitin, even under more drastic conditions.[7]

Although the Hakomori method[8] also was found effective in preparing alkali-chitin, the procedures involved were somewhat more complicated.

Table I. Elemental analyses of chitin and alkyl-chitins

Samples		C %	H %	N %	D.S.[a]
Chitin	Calcd	45.28	6.60	6.60	0[b]
	Found	44.96	6.50	6.57	
Ethyl-chitin	Calcd	47.31	6.54	6.27	0.4[c]
	Found	47.11	6.70	6.40	
Ethyl-chitin	Calcd	51.06	7.63	5.74	1.2[d]
	Found	50.96	7.36	6.17	
n-Butyl-chitin	Calcd	49.14	7.33	6.02	0.4[e]
	Found	48.55	6.87	6.32	
i-Butyl-chitin	Found	48.88	6.71	6.38	
t-Butyl-chitin	Found	48.75	6.87	6.40	
n-Amyl-chitin	Calcd	50.00	7.08	5.83	0.4[f]
	Found	49.01	7.18	6.08	
i-Amyl-chitin	Found	49.33	7.39	6.00	
t-Amyl-chitin	Found	49.26	7.41	6.05	

[a] D.S., degree of substitution per *N*-acetylglucosamine residue.

[b] Chitin. Calcd. for $C_8H_{13}NO_5 \cdot 1/2\ H_2O$.

[c] Ethyl-chitin. Calcd for $(C_8H_{13}NO_5 \cdot 1/2\ H_2O)_{0.6} + (C_{10}H_{17}NO_5 \cdot 1/2\ H_2O)_{0.4} = C_{8.8}H_{14.6}NO_5 \cdot 1/2\ H_2O$.

[d] Ethyl-chitin of 1.2 substituted was prepared from alkali-chitin and diethyl sulfate in *i*-propyl alcohol. Calcd for

$$(C_{10}H_{17}NO_5 \cdot 1/2\ H_2O)_{0.8} + (C_{12}H_{21}NO_5)_{0.2} = C_{10.4}H_{18.6}NO_{5.4}.$$

[e] Butyl-chitin. Calcd for $C_{9.6}H_{16.2}NO_5 \cdot 1/2\ H_2O$.

[f] Amyl-chitin. Calcd for $C_{10}H_{17}NO_5 \cdot 1/2\ H_2O$.

Alkylation of Chitin

Since the alkylation reaction was performed at low temperature to avoid deacetylation, the degree of alkylation per *N*-acetylglucosamine residue was not sufficient (around 0.4), independently of the molecular shape of the alkylation reagents, as shown by the results of elemental analyses in Table I. The low degree of substitution was probably due to the low reaction temperature and also the alignment of *N*-acetylglucosamine residue in the crystalline structure of chitin as suggested by Pearson *et al.*[9]

Differential scanning calorimetric measurements were made to estimate the extent of destruction of crystalline structure on the alkyaltion reaction. A slight depression in the energy of heat transition around 220°C was observed for the vacuum dried samples on increasing the bulkiness of butyl groups as shown in Figure 1. But large enhancement of heat transition energy was observed with an increase in the bulkiness of the alkyl group when the butyl chitin powder were equilibrated with moisture at 40°C. However, there was little difference in the

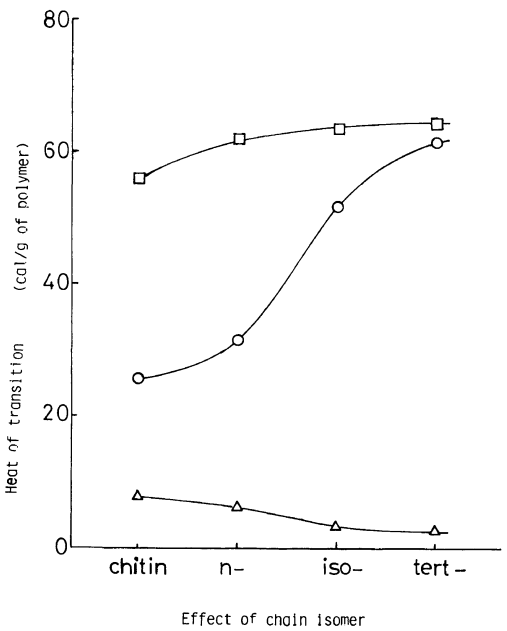

Figure 1. Heat transition of butyl-chitin powder: —△—, dried *in vacuo* at 40°C; —○—, equilibrated with moisture at 25°C; —□—, soaked with water of 25°C for 3 min. Energy of heat transition was estimated by reference to that of urea at heating rate of 5°C min^{-1}

Table II. Properties of chitin and alkyl-chitin fibers

Sample	Denier	Tenacity		Elongation		Knot strength	Young's modulus		Density	D. Sub.[c]
	d	g d^{-1}		%		g d^{-1}	g d^{-1}		g ml^{-1}	
		Dry[a]	Wet[b]	Dry	Wet		Dry	Wet		
Chitin	8.54	1.28	0.23	8.4	15.6	0.15	74.54	3.96	1.40	0
Chitin	4.16	1.39	0.33	3.6	10.1	0.64	88.42	19.42	1.40	0
Methyl-	3.73	0.60	0.06	4.2	3.5	0.20	45.80	—	1.37	0.4
Ethyl-	2.99	1.25	0.33	3.5	13.2	0.32	86.12	3.40	1.39	0.4
n-But-	4.15	0.79	0.10	1.0	7.1	0.11	108.26	—	1.43	0.4
i-But-	5.58	1.42	—	4.6	—	0.12	92.72	—	1.44	0.4
t-But-	3.56	0.92	—	3.5	—	0.15	86.52	—	1.41	0.4
n-Amyl-	4.23	0.92	—	3.5	—	0.38	88.70	—	1.46	0.4
i-Amyl-	3.69	1.20	—	3.5	—	0.13	102.79	—	1.41	0.4
t-Amyl-	2.53	0.82	0.09	2.5	17.3	0.48	89.57	—	1.42	0.4

[a] Dry: 20°C, 65% R.H.
[b] Wet: 20°C, 100% R.H.
[c] D. Sub.: Degree of substitution estimated from elemental analysis.

Table III. Chitin and alkyl-chitin fiber spacings

Samples	Spacings Å	
Chitin	3.44—3.59	m, q
	3.72—3.88	m, q
	4.59—4.98	m, q
	7.70—12.59	m, q
n-Butyl-chitin	3.53—3.72	q
	4.67—4.91	m
	4.74—5.12	q
	5.38—5.60	q
i-Butyl-chitin	3.53—3.65	q
	4.67—4.96	m
	4.74—5.00	q
t-Butyl-chitin	3.53—3.65	q
	4.62—5.38	m
	4.73—5.03	q
	5.32—5.51	q

Table IV. Size of fiber unit cells calculated from X-ray diffraction patterns

Fibers	Axis		
	a (Å)	b (Å)	c (Å) Fiber repeat
Chitin	4.7	10.5	10.3
n-Butyl-chitin	4.8	10.6	10.7
i-Butyl-chitin	4.9	10.9	10.7
t-Butyl-chitin	5.0	11.0	10.9

energy of heat transition among the alkyl-chitins on the wet state, though the transition temperature was depressed to around 151°C by the presence of water. Since the increase in heat transition energy depends on the presence of water molecules, the partial destruction of the crystalline structure of chitin seems to be induced by alkylation and it is possible that the resulting amorphous part easily absorbs water. The enhancement of the solubility of alkyl-chitin toward formic acid also seems to suggest an increase in the amorphous part of the chitin.

Regeneration of Alkyl-Chitin Fibers

The alkyl-chitin fibers were prepared by the wet spinning of alkyl-chitin-formic acid solution into ethyl acetate according to the method for preparing acetyl-chitin fibers.[3] The water bath could not be used for stretching the coagulated alkyl-chitin fibers, since they quickly become fragile on absorbing water. Hence, the stretching procedure was carried out in the air to obtain well oriented fibers.

The properties of the alkyl-chitin fibers are listed in Table II along with those of chitin fibers whose properties did not improve with alkylation.

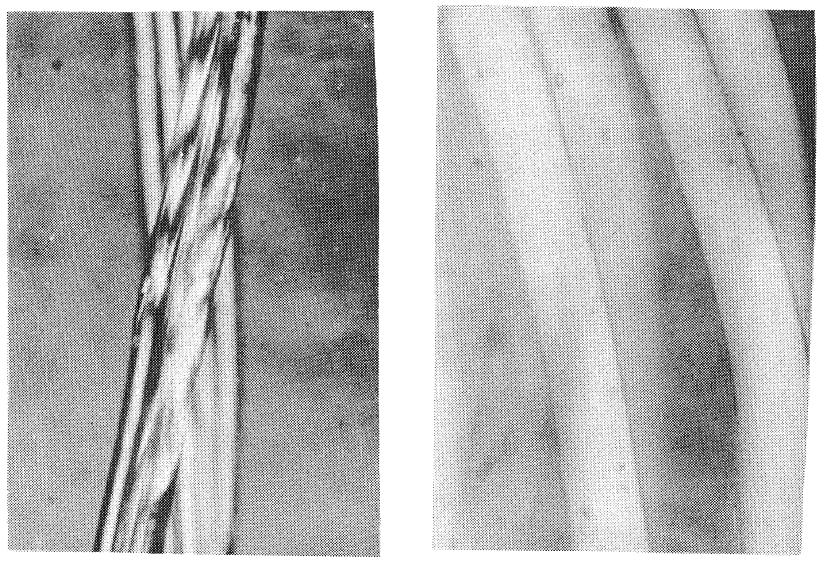

Figure 2. Microscopic pictures of *n*-butyl-chitin fibers. Fiber pictures were taken before and after contact with water at 25°C for 3 min. Diameters of dried or wet fibers were measured from these pictures.

However, the orientation of butyl-chitin fibers was generally better than that of chitin fibers from X-ray diffraction patterns whose spacings are listed in Table III along with those for chitin fibers. Although the amyl-chitin fibers showed a tendency similar to that of butyl-chitin fibers, the orientations of methyl- and ethyl-chitin fibers were nearly identical to that of the chitin fibers.[1,10-13]

The increase in the spacings of the regenerated alkyl-chitin fibers corresponds to a small extent to the bulkiness of attached alkyl group as shown in Table III. This might be possibly the result of steric hindrance of the butyl group on the reformation of rigid crystalline structure in the chitin molecule and thus the linear polysaccharide chain might slide easily to orient by stretching. The unit cells in the regenerated butyl-chitin fibers calculated from the values in Table III, seem to support above speculation in view of the slight expansion in the size of unit cell with increase in the bulkiness of the butyl group as shown in Table IV. The loose packing of molecules in the alkyl-chitin fibers did not change even after being subjected to such treatment as heating *in vacuo* or annealing.

The regenerated alkyl-chitin fibers quickly swell on contact with water as shown by the microscopic observations in Figure 2. The diameters of the dry and wet fibers were measured from microscopic pictures and then the degree of swelling was calculated from the ratio of the fiber cross sections or

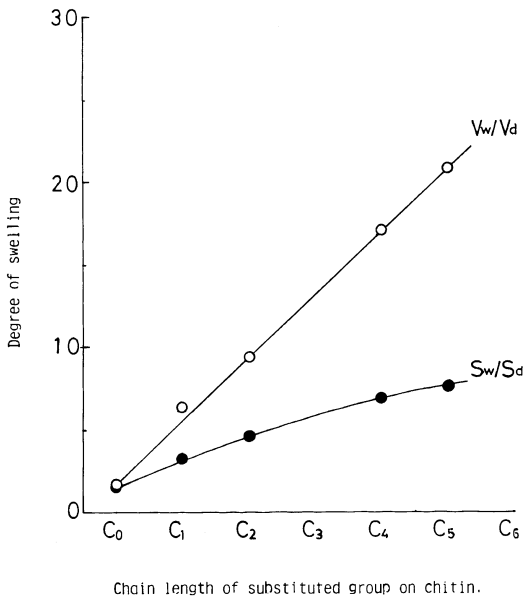

Figure 3. Dependence of the swelling on the chain length of linear alkyl groups. Degree of swelling is expressed as V_w/V_d or S_w/S_d. V, volume of fiber; S, cross section of fiber.

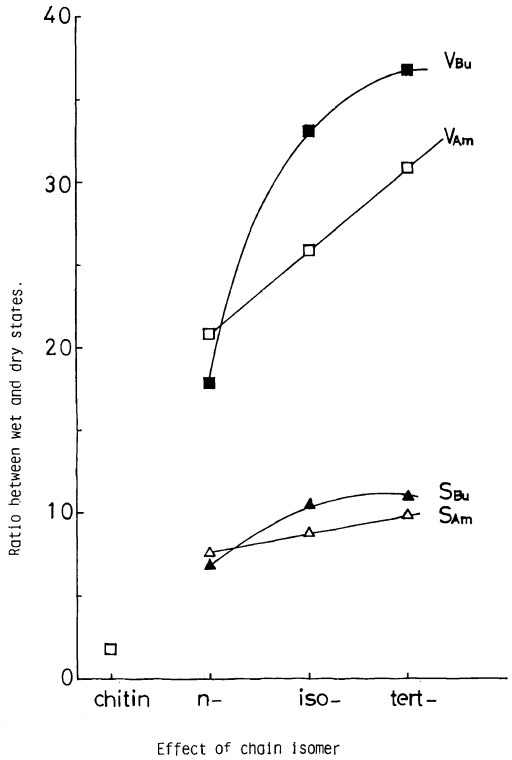

Figure 4. Dependence of degree of swelling on bulkiness of alkyl groups. *V* and *S* are the volume and cross section of the fibers, respectively.

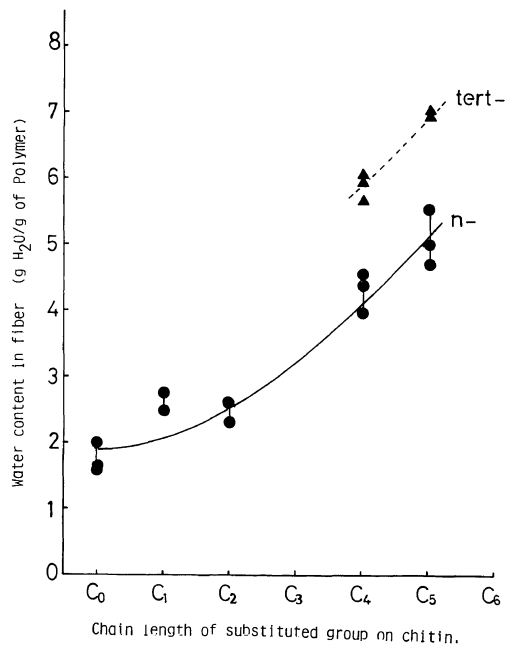

Figure 5. Dependence of fiber wettability on chain length and bulkiness of alkyl groups. Water analysis of soaked fibers was carried out using the method of Kirl-Fischer with an automatic analyzer at 20°C.

volumes in the dry and wet states. The degree of swelling tended to increase with the increment in the chain length of the introduced alkyl groups as shown in Figure 3. The swelling of the fibers was also found to depend on the bulkiness of the alkyl group as shown in Figure 4. It was also noted that the complete reformation of chitin molecule might be inhibited by the presence of an alkyl group. The amount of water absorbed on alkyl-chitin fibers was measured by the Kirl-Fischer water analysis and the results are shown in Figure 5. The absorption of water depended on the bulkiness and chain length of the alkyl groups. The biregringence under the polarized light, observed for the original alkyl-chitin fibers, was disappeared on contact with water and a more dispersed X-ray diffraction pattern was obtained on redrying the swollen fibers. The disordered alignment of the alkyl-chitin molecule seems to take place by contact with water.

^{13}C and ^{1}H NMR Studies

The disruption of the glycoside linkage and the formylation of an *N*-acetylglucosamine residue are considered to occur during the NMR mesurements, because a relatively higher temperature is required to obtain better peak separation in the deuterated formic acid. The detection of chemical shift was thus fairly difficult for samples of low degree substituion. Hence, a highly substituted chitin, 1.2 ethylated chitin solution in deuterated formic acid, was subjected to ^{13}C NMR measurement to detect the initial alkylation site on the *N*-acetylglucosamine residue. The NMR spectrum of 1.2 ethyl-chitin is shown in Figure 6 along with that of the chitin solution in deuterated formic acid. The peak area due to the ethoxy methyl group (14.7 ppm) of 1.2 ethyl-chitin was estimated to be 10—20% larger than that of acetamide group (22.7 ppm) when both peak areas were compared with each other by the gated decoupling method. Since the degree of ethylation estimated from elemental analysis was around 1.2, the comparison of peak areas by the gated decoupling method allowed a reliable estimation of the degree of substitution on the

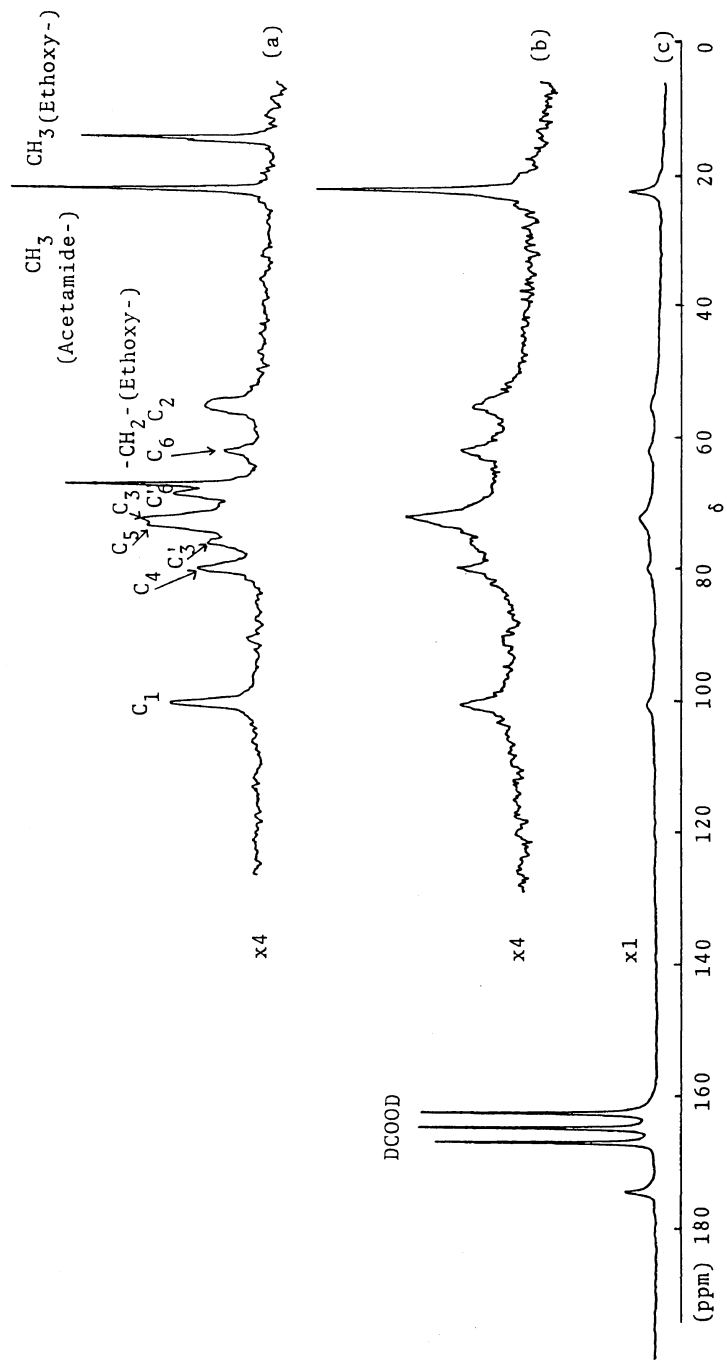

Figure 6. ^{13}C NMR spectra at 15 MHz of chitin (b, c) and ethyl-chitin (a) in DCOOD at 45°C. C′: signals of modified carbons: spectral width, 3002 Hz; pulse angle, 90° (10 μs); repetition time, 2.0 s; 8 K data memory; 21600 accumulations.

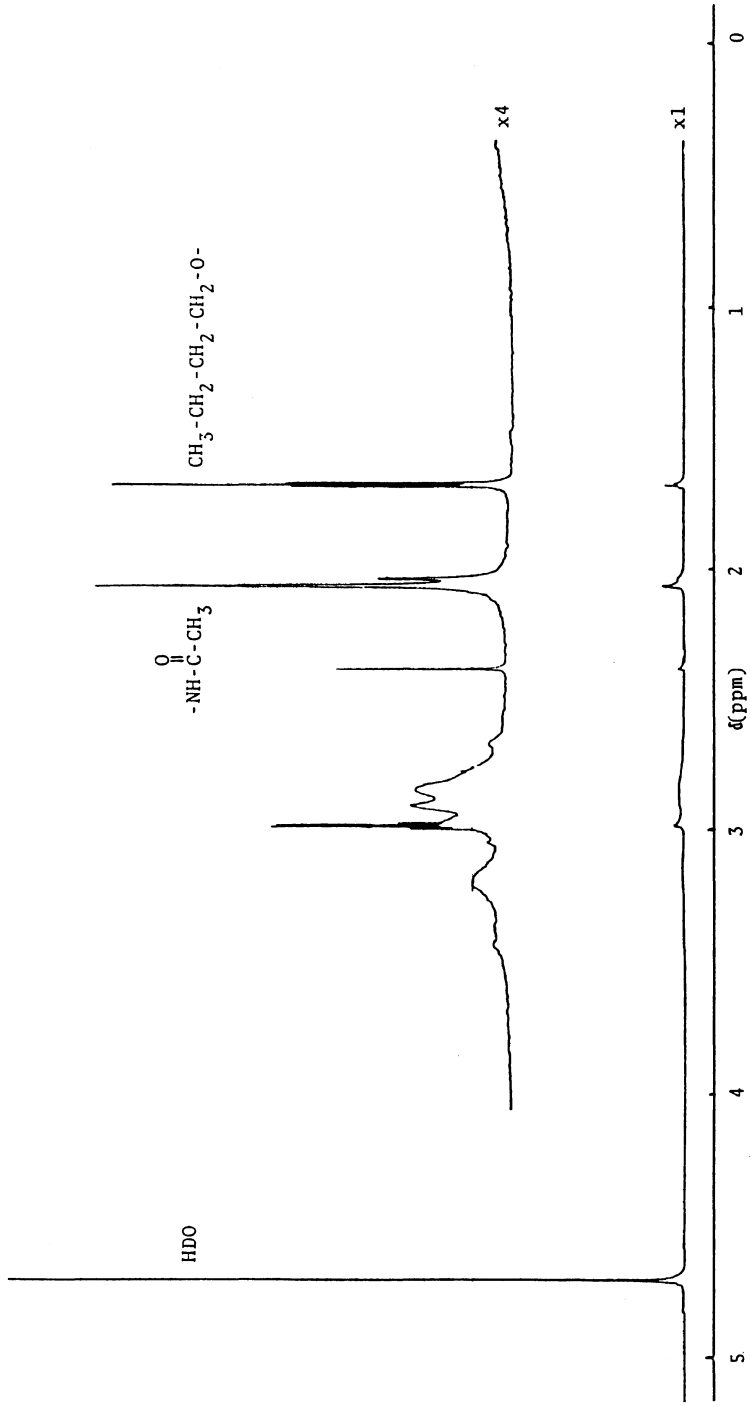

Figure 7. ^{1}H NMR spectrum at 400 MHz of *n*-butyl-chitin in DCOOD at 45°C: spectral width, 6002 Hz; pulse angle, 90° (10 μs); repetition time, 10 s; 16 K data memory; 4 accumulations.

highly substituted chitin. Since the signal of the carbon attached to the hydroxyl group in the glucose is known to shift to a lower magnetic field (8—10 ppm) when blocked with methyl group,[14,15] it may be suggested that the signals at 69.4 ppm corresponded to the ethoxylated carbon at C_6 and 76.8 ppm to that at C_3 position of *N*-acetylglucosamine residue. The signals of 62.6 ppm and 74.0 ppm are assigned to those for the unsubstituted C_6 and C_3, respectively, according to the ^{13}C NMR studies for glucose and the solid chitin.[16] The signal at 67.7 ppm was also assigned to the methylene of ethoxy group. The assignment for each signal is shown in Figure 6-a. The intensity ratio of the shifted signal to the unshifted one was approximately 2 : 1 for C_6. The intensity ratio for C_3 was approximately 1 : 3.8, although the C_3 and C_5 signals appeared in position very close each other, and C_3' (substituted) peak was too small for comparison. Thus 67% of hydroxyl group at C_6 and 21% at C_3 position were suggested to be substituted with 1.2 ethylation. There was no indication of chemical shift due to the substitued C_3 on the 0.4 butyl-chitin. Therefore, the initial alkylation site may be assumed to be a primary alcohol at the C_6 position. According to Pearson *et al.*[9] two linear chain of poly-*N*-acetylglucosamine are arranged in reverse direction to form a crystalline structure of β-chitin. The rigid crystalline structure of β-chitin is contributed by the intermolecular and intramolecular hydrogen bonds. The intramolecular hydrogen bond is participated by an acetamide group at C_2 and a hydroxyl group at C_3 positions. The intermolecular hydrogen bond is formed between hydroxyl groups at C_6 and C_3 through a water molecule. The hydroxyl group at C_3 position is likely covered both with intra- and intermolecular hydrogen bonds. The present work shows the substitution to be independent of the molecular shape of the alkylating reagents. The degree of alkylation was around 0.4, indicating that a hydroxyl group at the C_6 of each of the 2—3 *N*-acetylglucosamine residues was substituted. On taking into account the fact that the hydroxyl group at C_3 is protected by two kinds of hydrogen bonds involving water molecule, the prior alkylation of the hydroxyl group at C_6 position and the low degree of alkylation on the single preparation can be reasonably explained. The loose packing of alkyl-chitin molecule may also be attributed to the disruption of the intermolecular hydrogen bond by the alkylation reaction, and along with steric hindrance, the attached alkyl group may also hinder the reformation of the rigid crystalline structure. This assumption seems to be supported by the brittle properties of wet fibers, quick swelling by soaking in water and the depression of the energy of heat transition on the alkyl-chitin fibers.

The degree of substitution was also estimated from ^1H NMR measurement of *n*-butyl-chitin-DCOOD solution as shown in Figure 7. The area ratio of the signals of *n*-butoxy methyl group (1.67 ppm) to acetamide methyl group (2.06 ppm) was 0.36 which is almost identical to that from elemental analysis.

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