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Bromination of regenerated chitin with *N*-bromosuccinimide and triphenylphosphine under homogeneous conditions in lithium bromide–*N*,*N*-dimethylacetamide

How Tseng, Ken-ichi Furuhata, Munenori Sakamoto

Department of Organic and Polymeric Materials, Faculty of Engineering, Tokyo Institute of Technology, O-okayama, Meguro-ku, Tokyo 152, Japan

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Abstract

Chitin regenerated from LiCl–*N*,*N*-dimethylacetamide (DMA) was found to dissolve in 10 g/dL LiBr–DMA. The bromination of the regenerated chitin proceeded to a large extent (DS by bromine up to 0.94) with equimolar amounts of *N*-bromosuccinimide and triphenylphosphine under homogeneous conditions in LiBr–DMA at 50–90°C. ^{13}C NMR spectroscopy of brominated products and GLC-MS analysis of their hydrolyzates showed that the bromine substitution took place regioselectively at C-6 of the chitin repeating units. Polymer chain scission occurred to some extent during the bromination, more extensively at higher temperatures with higher concentrations of reagents.

1. Introduction

The usefulness of halodeoxycelluloses in cellulose chemistry has been well established [1,2]. Likewise, halodeoxychitins are expected to be useful for further chemical modifications. There are a few studies on the halogenation of chitin. Kurita and his coworkers [3] reported the preparation of iododeoxychitin of DS 0.58 from *O*-tosylchitin. We reported recently [4] the regioselective chlorination of chitin with *N*-chlorosuccinimide (NCS) and triphenylphosphine (Ph_3P) under homogeneous conditions in LiCl–*N*,*N*-dimethylacetamide (DMA). Chlorodeoxychitins with DS up to ≈ 1.0 were easily prepared under appropriate conditions.

Bromine is a better leaving group than chlorine, and the C–Br bond is higher in stability than the C–I bond, as was observed in the nucleophilic substitutions of

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halodeoxycelluloses [5]. Some undesirable eliminations might occur during the nucleophilic substitutions of iododeoxychitins. Bromodeoxychitins should therefore be more useful intermediates than chlorodeoxychitins or iododeoxychitins for the preparation of chitin derivatives. The bromination of chitin has not yet been reported.

Regenerated chitin was found to dissolve in the LiBr–DMA solvent system and this was suitable for the bromination. In this paper, the bromination of chitin in LiBr–DMA is described as part of a series of studies on the halogenation of polysaccharides.

2. Results and discussion

Solvent system for chitin, containing LiBr.—In our previous study [4], the chlorination of chitin was effected with NCS and Ph_3P in LiCl–DMA, where the excess chloride ions participated in the chlorination. For the bromination of chitin under homogeneous conditions, solvent systems containing bromide ions are expected to be advantageous. We therefore examined the LiBr–DMA system for the dissolution of chitin. This solvent system was found to dissolve cellulose [6], but unexpectedly the purified chitin did not dissolve in LiBr–DMA, and was only swollen.

The insolubility of chitin in common solvents is attributable to the strong intermolecular hydrogen bonds of chitin, and breakage of the hydrogen bonds is essential for the dissolution of chitin. Vincendon [7] proposed a mechanism of dissolution of chitin in LiCl–DMA based on ^1H NMR measurements, in which chloride ions interacted with labile protons (OH or NH) thus promoting the breakage of intermolecular hydrogen bonds in chitin. The principal concept adopted is the same as proposed for the dissolution of cellulose in LiCl–DMA [8]. DMA is known to interact strongly with metal cations to leave non-solvated anions. The order of nucleophilicity of non-solvated halide ions decreases in the order, $Cl^- > Br^- > I^-$ [9]. The insolubility of the purified chitin in LiBr–DMA may be ascribed to the weaker nucleophilicity of bromide ion as compared with chloride ion, making it difficult to break the hydrogen bonds in the purified chitin.

Chitin samples with decreased crystallinities are expected to show higher solubility. The chitin regenerated from its LiCl–DMA solution by precipitation in acetone was found to dissolve in 10 g/dL LiBr–DMA at 70°C to give a clear solution. Figure 1 shows the wide-angle X-ray diffraction (WAXD) profiles of the purified and the regenerated chitins. The degree of crystallinity of the regenerated chitin (Fig. 1b) was about 0.58, lower than that (about 0.65) of the original purified chitin (Fig. 1a). The decrease in hydrogen bonding by regeneration was also indicated by the IR spectrum of the sample in the amide A and B regions. The IR data are discussed later, together with those of bromodeoxychitins. The possibility of partial chain scission of the regenerated chitin during dissolution in LiBr–DMA was checked by viscometry. Figure 2 shows that the reduced viscosity of the regenerated chitin in 10 g/dL LiBr–DMA does not decrease on heating at 90°C even after 24 h. This finding shows that little chain scission occurs during the dissolution process.

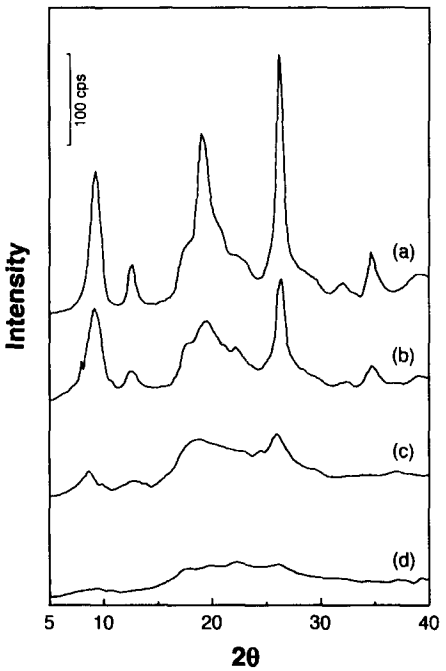

Fig. 1. WAXD profiles of (a) purified chitin, (b) regenerated chitin, and bromodeoxychitin with DS of (c) 0.42 and (d) 0.90.

Synthesis of bromodeoxychitins.—The concentration of the regenerated chitin (*N*-deacetylation, 5.7% based on elemental analysis) in the reaction medium was fixed at 0.5 g/dL (concentration of the repeating unit (PRU) of chitin, 25 mmol/L) and that of LiBr to 10 g/dL (1.15 mol/L) in the present bromination. Equimolar amounts of *N*-bromosucinimide (NBS) and Ph_3P were used and their concentrations are expressed as the molar ratios to PRU of chitin ([NBS · Ph_3P]/[PRU]). The bromination of chitin

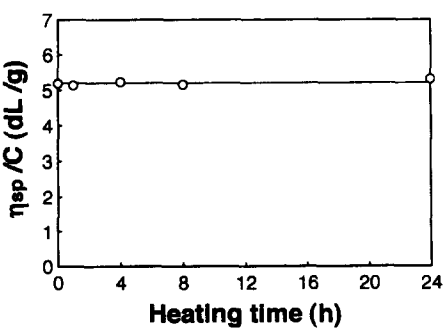

Fig. 2. Reduced viscosity of regenerated chitin as a function of time of heating at 90°C measured in 10 g/dL LiBr–DMA at 40°C (C, 0.23 g/dL).

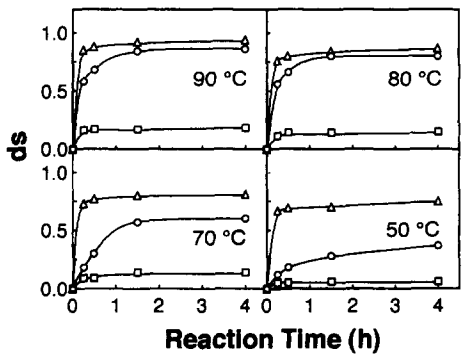

Fig. 3. Bromination of chitin under various conditions: [NBS·Ph_3P]/[PRU]; 4 (□), 6 (○) and 8 (△).

did not proceed appreciably at a reagent molar ratio equal to or below 3, nor did it proceed much even at higher molar ratios of reagents at reaction temperatures below 40°C. However under appropriate conditions, the bromination of chitin proceeded to an appreciable extent, and white to pale-yellow powdery products were obtained with recoveries over 80%. Figure 3 shows the DS values of the products as a function of bromination time at 50–90°C and at the reagent molar ratios of 4–8. The DS values of the products levels off in 0.5–2 h. The leveled-off DS values are dependent on the bromination conditions. At a low molar ratio of reagents ([NBS · Ph_3P]/[PRU] = 4), the value of the leveled-off DS remains low and is only 0.2 at 90°C. At higher molar ratios of reagents (6 or 8), the leveled-off DS values are 0.85 or higher, depending on the reagent molar ratio and the reaction temperature. The highest DS value attained was 0.94 and samples having DS values > 1.0 could not be obtained under these conditions. These findings are very similar to those observed for the chlorination of chitin under homogeneous conditions [4]. The chlorination of cellulose with NCS and Ph_3P in LiCl–DMA took place at C-6 initially, and then further at C-3 whereas the bromination of cellulose with NBS and Ph_3P took place only at C-6 [5]. In contrast,the C-3 hydroxyl groups of chitin were unreactive even for the chlorination with NCS and Ph_3P [4]. This indicated that the apparent reactivity of NBS and Ph_3P is similar to that of NCS and Ph_3P in the case of chitin.

Structure of repeating units of bromodeoxychitins.—Bromodeoxychitins dissolved in 35% HCl and they were completely hydrolyzed to monosaccharides when heated for 6 h at 80°C, as in the case of chitin and chlorodeoxychitins [4]. The hydrolyzates were analyzed by GLC and GLC-MS as volatile *N,O*-trifluoroacetyl (TFA) derivatives. Figure 4 shows GLC traces for the hydrolyzates of bromodeoxychitins of different DS values together with that of chitin. Chitin gives only two anomeric peaks for the *N,O*-TFA derivative of glucosamine (GlcN) whereas bromodeoxychitins give anomeric peaks for the *N,O*-TFA derivatives of GlcN and 6-bromo-6-deoxyglucosamine (GlcN–6Br), all in the pyranose form. Figure 4 clearly shows that the bromination takes place regioselectively at C-6. As with the cellulose analogue, the hydrolysis of bromodeoxychitins proceeded uneventfully to give the constituent saccharides.

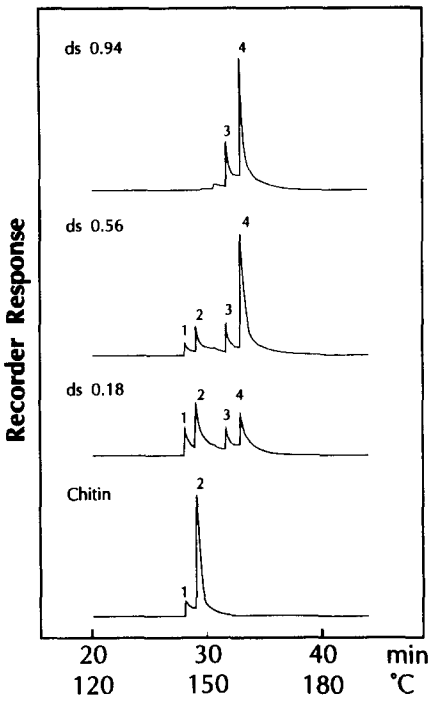

Fig. 4. GLC traces of hydrolyzates of chitin and bromodeoxychitins as *N,O*-TFA derivatives: (1) and (2), GlcN*p*; (3) and (4), GlcN*p*–6Br.

The structure of GlcN–6Br was determined on the basis of its MS fragmentation pattern as the *N,O*-TFA derivative. Data are summarized in Table 1, together with those for GlcN and 6-chloro-6-deoxyglucosamine (GlcN–6Cl) [4]. The nomenclature of fragment ions proposed by Kochetkov and Chizhov [10] is used in the table. The fragmentations are very similar to each other. The assignment of some of the fragment ions for GlcN–6Br such as A_1, was confirmed by the isotopic pattern due to bromine substitution (1:1 doublet). The E series ions of GlcN–6Br indicate its pyranose structure. The E_1 ion of GlcN-6Br (singlet at m/z 533), formed by the loss of the CH_2Br moiety from the molecular ion, indicates the bromine substitution to be at C-6.

The structure of bromodeoxychitins was confirmed by the measurement of ^{13}C NMR spectra in trifluoroacetic acid-*d* of the TFA derivatives. Figure 5 shows the ^{13}C NMR spectra of bromodeoxychitins having different DS values, together with those of chitin and chitosan. Because of the low solubility of bromodeoxychitins, even after trifluoroacetylation, and of the low frequency of the spectrometer used, the ^{13}C NMR spectra of the samples had a low level of signal-to-noise ratio. The C-6 carbon signals of chitin around 69 ppm become weaker for the bromodeoxychitin sample of DS 0.50 and almost disappear for the sample having a DS value of 0.87. A new absorption appears at 32 ppm for bromodeoxychitins, and the relative intensity is higher for the sample having DS of 0.87. The chemical shift of this new signal is close to that reported for C-6 of

Table 1
Mass fragmentation patterns of glucsamine as *N,O*-trifluoroacetyl derivatives

Fragment [a]	Peak 1,2 (GlcN*p*) [b]		(GlcN6Cl*p*) [c]		Peak 3,4(GlcN6Br*p*) [d]	
	m/z	r.a. [e]	*m/z* [f]	r.a. [e]	*m/z* [g]	r.a. [e]
M^+	659	0.03	581(d)	0.00	625(d)	0.28
A_1	546	2.65	468(d)	2.50	512(d)	1.34
A_2	432	1.33	432(s)	0.10	432(s)	0.36
			354(d)	1.30	398(d)	0.66
A_3	318	17.57	318(s)	0.20	318(s)	2.21
			240(d)	28.00	284(d)	13.68
E_1	532	0.83	532(s)	2.00	532(s)	0.19
E_2	418	0.32	418(s)	0.10	418(s)	0.35
E_3	304	4.68	304(s)	2.80	304(s)	1.54
C_2	404	13.06	404(s)	4.60	404(s)	32.24
			327(d)	12.70	370(d)	1.77
F_1	265	21.62	265(s)	45.60	265(s)	41.69
CF_3CO	97	18.01	97(s)	14.90	97(s)	15.15
C_5H_5O	81	9.46	81(s)	7.00	81(s)	7.98
CF_3	69	100	69(s)	100	69(s)	100

[a] Nomenclature for fragment ions, see text and ref. 10. [b] Peak 1,2 for chitin. [c] See ref. 4. [d] Peak 3,4 for bromodeoxychitin. [e] r.a., relative abundance(%); [f] Mass number for GLcN6CL are based on ^{35}Cl: d, 3:1 doublet at m and m+2; s, singlet. [g] Mass number for GLcN6Br are based on ^{79}Cl: d, 1:1 doublet at m and m+2; s, singlet.

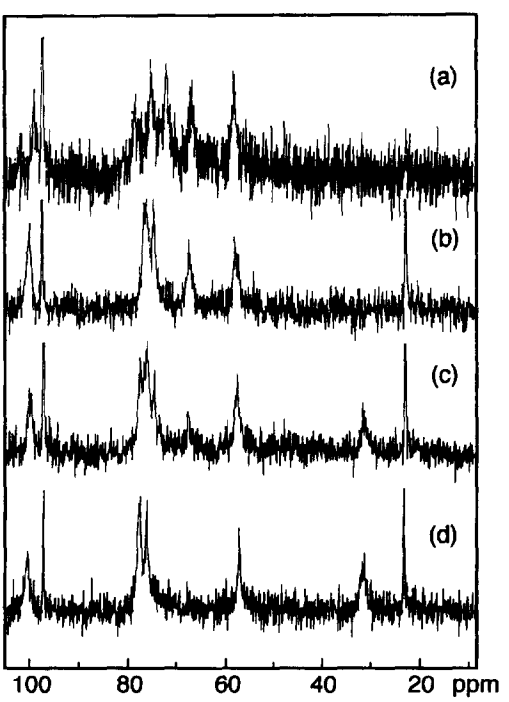

Fig. 5. ^{13}C NMR spectra in trifluoroacetic acid-*d* at 22.53 MHz and 40°C: (a) chitosan, (b) chitin; bromodeoxychitin with DS of (c) 0.48; (d) 0.87. The signal near 98 ppm is the solvent signal.

6-bromo-6-deoxysaccharides [11,12]. The result clearly indicates that the replacement of hydroxyl groups with bromine took place only at C-6. The chemical shifts of carbons other than $C\text{-}6^{Br}$ are close to those observed for chlorodeoxychitins as TFA derivatives [4]. Chitin and the two bromodeoxychitins give the $COCH_3$ signal around 23 ppm. The intensity of this signal relative to that of the C-1 signal is almost equal for the three samples. The degrees of *N*-deacetylation of the regenerated chitin and bromodeoxychitins determined by elemental analysis were in the range 4.3–6.8%. These findings show that, as in the case of chlorodeoxychitins [4]. neither N → O acetyl migration nor amide exchange took place during trifluoroacetylation and dissolution into trifluoroacetic acid.

Some properties of bromodeoxychitins.—The solubilities of the bromodeoxychitin samples were examined. Bromodeoxychitins were immediately soluble in formic acid without repeated freezing and defreezing treatments necessary for dissolution of chitin [13]. They were insoluble in such common organic solvents as MeOH, EtOH and acetone, but the samples having DS values above 0.5 swelled in such aprotic solvents as CH_3Cl, pyridine, *N*-methyl-2-pyrrolidone, DMA, and hexamethylphosphoric triamide. Samples having DS values > 0.7 were soluble in Me_2SO. All of the bromodeoxychitins were soluble in 10 g/dL LiBr–DMA or 5 g/dL LiCl–DMA, and the solubility tended to increase with increasing DS. The immersion of LiBr–DMA solutions cast on glass plates into EtOH gave transparent films.

The increased solubility may reasonably be ascribed to the lowered crystallinity (reduction in hydrogen bonding), possible main-chain scission during the bromination, and/or the change in the chemical structure induced by bromination. The replacement of C-6 hydroxyl groups by bromine will disrupt the hydrogen-bonded structure in which C-6 hydroxyl groups are involved. The WAXD patterns of bromodeoxychitins shown in Fig. 1 clearly indicate a decrease in crystallinity. The degrees of crystallinity estimated for the samples (0.11–0.52) were lower than that of the regenerated chitin (about 0.58). This is considered to be due, at least in part, to the replacement of C-6 hydroxyl groups by bromine atoms (random replacement is expected under homogeneous conditions), resulting in a decrease in the hydrogen bonding.

Figure 6 shows the IR spectra of chitin samples in the amide A and B regions. The amide A band for secondary amides, usually appearing near 3265 cm^{-1}, shifts to higher wavenumber and the amide B band near 3110 cm^{-1} shifts to a lower wavenumber, with decreasing hydrogen bonding [14,15]. This tendency is clearly seen in the spectra shown in Fig. 6. The amide A band shifts slightly but steadily to higher wavenumber on going from the purified chitin to the regenerated chitin and bromodeoxychitins with increasing DS while the amide B band showed the opposite tendency. This finding shows that the hydrogen bonding in the samples decreased sequentially from the purified chitin to the bromodeoxychitin having DS 0.94.

The specific rotation of chitin is reported to be $-193°$ in $CaCl_2 \cdot 2H_2O$–MeOH [16] and $-19°$ in 35% HCl [17]. The specific rotations of chitin and bromodeoxychitins were measured in HCO_2H (Fig. 7). The specific rotation changes continuously from 4.9° for the regenerated chitin to $-25.2°$ for the bromodeoxychitin having d.*s* of 0.85.

Figure 8 shows the recoveries (based on the repeating unit) of bromodeoxychitins obtained under various bromination conditions. When the reaction is carried out at a

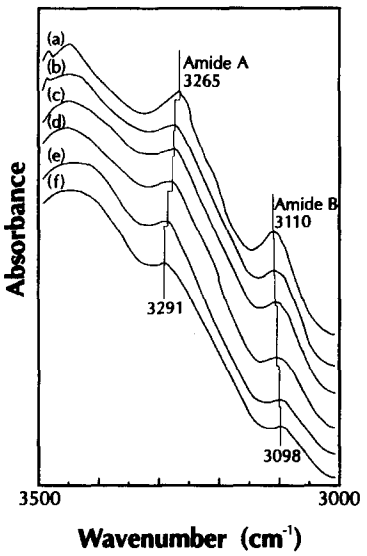

Fig. 6. IR spectra of chitin and bromodeoxychitins in amide A and B regions: (a), purified chitin; (b), regenerated chitin; bromodeoxychitins with DS of (c), 0.15; (d), 0.36; (e), 0.74; (f), 0.94.

high molar ratio of reagents ([NBS · Ph_3P]/[PRU] = 8 or 10), the recovery falls with increasing reaction time, even after the DS has leveled off, and the tendency is more remarkable at higher reaction temperatures. When chitin is brominated at the lower reagent molar ratio of 4 at 50°C, the recovery is almost quantitative throughout the reaction. The decrease in recovery under more severe reaction conditions suggests that products of low molecular weight were formed through the molecular chain scission during the bromination, and were lost during the isolation of brominated products.

Bromodeoxychitins were analyzed by GPC after trifluoroacetylation. Monodispersed polystyrene standards were used for calibration, and the apparent dp values were

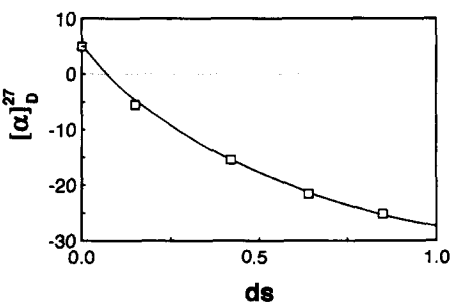

Fig. 7. Specific rotations of chitin and bromodeoxychitins in HCO_2H at 27°C (c 1.0).

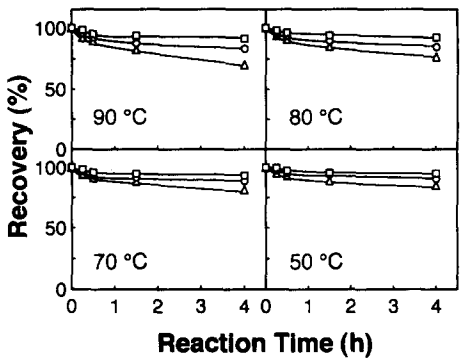

Fig. 8. Recoveries of bromodeoxychitins prepared under various reaction conditions: [NBS·Ph3P]/[PRU]; 4 (□), 6 (○) and 8 (△).

calculated based on the apparent number-average molecular weights. Figure 9 shows the apparent dp distribution curves for bromodeoxychitin samples. The peak maximum shifts to the lower side of dp with increasing reaction time (Fig. 9a), reaction

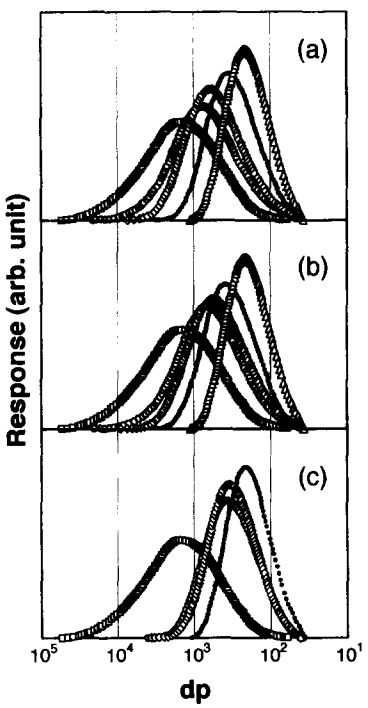

Fig. 9. Apparent dp distributions of chitin and bromodeoxychitins: (a) effect of reaction time; chitin (□) and bromodeoxychitins prepared at [NBS·Ph3P]/[PRU] of 8 at 90°C for 15 min (○), 30 min (◇), 1.5 h (○) and 4 h (△): (b) effect of reaction temperature; bromodeoxychitins prepared at [NBS·Ph3P]/[PRU] of 8 for 4 h at 50°C (○), 70°C (◇), 80°C (○) and 90°C (△): (c) effect of [NBS·Ph3P]/[PRU]; bromodeoxychitins prepared for 4 h at 90°C at [NBS·Ph3P]/[PRU] of 4 (○), 6 (◇) and 8 (○).

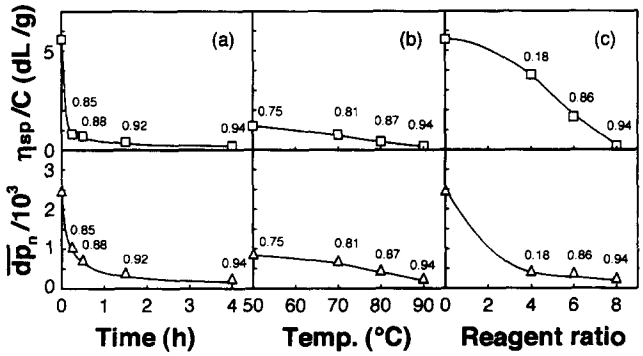

Fig. 10. Relationship between reduced viscosity and apparent dp distribution for chitin derivatives: (□), viscosity; (△) dp: (a) effect of reaction time at [NBS·Ph3P]/[PRU] of 8 at 90°C; (b) effect of reaction temperature at [NBS·Ph3P]/[PRU] of 8 for 4 h; (c) effect of [NBS·Ph3P]/[PRU] for 4 h at 90°C. Reduced viscosities were measured in 10 g/dL LiBr–DMA at 40°C (C, 0.1 g/dL). Numbers on the symbols represent the ds values of the samples.

temperature (Fig. 9b) and reagent molar ratio (Fig. 9c). The decrease in apparent dp for bromodeoxychitins is more significant as compared with that for chlorodeoxychitins [4], and this is probably because of more extensive-degradation by the stronger oxidant NBS than by NCS [18].

The reduced viscosities of the bromodeoxychitins used for the GPC analysis were measured in 10 g/dL LiBr–DMA. Figure 10a shows that both viscosity and dp of the reaction product decrease rapidly at the beginning of the bromination, even in 15 min, at [NBS · Ph_3P]/[PRU] of 8 at 90°C. Figure 10b shows that the values of reduced viscosity and dp of the bromodeoxychitin decrease with increasing temperature during in 4 h at an [NBS · Ph_3P]/[PRU], ratio of 8. Figure 10c shows the effect of reagent molar ratio in the bromination for 4 h at 90°C. The viscosity of the sample obtained at the reagent molar ratio of 4 (ds, 0.18) does not decrease appreciably as compared with that of the regenerated chitin whereas its dp is significantly lowered to a value close to those of samples having DS ≈ 0.9. This finding shows that the chain scission occurred even under mild bromination conditions and that the replacement of C-6 hydroxyl groups with bromine resulted in a decrease in reduced viscosity.

3. Conclusions

A novel solvent-system for regenerated chitin has been found, 10% (w/v) LiBr–DMA, that does cause chain scission during the dissolution. Bromodeoxychitins were prepared with NBS and Ph_3P in this solvent system, and DS of 0.94 could be attained under appropriate conditions. Hydroxyl groups at C-6 of the dissolved chitin were regioselectively replaced with bromine. Bromodeoxychitins are expect to be more suitable than chlorodeoxychitins for the introduction of functional groups through nucleophlilic substitutions.

4. Experimental

Materials.—Chitin, prepared from shrimp, was kindly supplied by Katokichi Co., Ltd. It was purified by the method of Tokura [19]. The regenerated chitin (the degree of *N*-deacetylation, 5.7%; molecular mass of PRU, 200.79) was prepared from the purified chitin by dissolution in 5 g/dL LiCl–DMA and precipitation from an excess of acetone. *N*-Bromosuccinimide (NBS) and Ph_3P were purified by recrystallization from distilled water and EtOH, respectively. Lithium bromide was heated at 185°C under diminished pressure. *N,N*-Dimethylacetomide (DMA) and DMF were distilled over CaH_2 under diminished pressure in N_2 stream and stored over Linde Type 4A molecular sieves under N_2. All other chemicals were used without further purification.

Bromination of chitin.—All procedures for the dissolution and bromination of chitin were carried out under N_2. To a flask containing 40 mL of DMA, 8 g of LiBr was added. The mixture was heated for 1 h at 75°C with stirring, cooled to room temperature, and 0.4 g of regenerated chitin was added. The temperature was raised to 75°C again, and the mixture was stirred for 24 h to give a clear homogeneous solution. In a typical bromination, given amounts of NBS and Ph_3P (both dissolved in appropriate amounts of DMA) were added quickly in this order to the solution just mentioned, under cooling with ice water. The final volume of DMA was set at 80 mL. The solution was then stirred for $\approx$ 15 min at room temperature and kept at a given temperature for the required time with stirring. The resultant brown solution was poured into 800 mL of acetone. Precipitates were collected by centrifugation, washed with acetone and then with MeOH, and treated with a Na_2CO_3 solution (pH, 11.4) at room temperature overnight. The products faded in color upon this treatment. They were washed with water and dialyzed against distilled water for 5 days. After lyophilization, bromodeoxychitins were obtained as light-yellow powders. Crude bromodeoxychitins produced at high molar ratios of reagents often showed weak IR absorption at 1745 cm^{-1} assignable to ester carbonyl, which disappeared after the alkali treatment. The bromine contents were determined by an oxygen-flask combustion method [20] from which DS values of the bromodeoxychitins were calculated. The samples were insoluble in 40 g/dL NaOH and the titration method used for chitin and chitosan [21] could not be applied. Therefore the degrees of *N*-deacetylation of bromodeoxychitins were calculated from the data of elemental analysis. IR (KBr disc) of bromodeoxychitin of DS 0.94: 3458 (ν_{O-H}), 3291 (amide A), 3098 (amide B), 1659 (amide I) and 1556 cm^{-1} (amide II). ^{13}C NMR (CF_3COOD) of bromodeoxychitin of DS 0.48: δ 23.1 ($COCH_3$), 31.9 (C-6 Br), 58.2 (C-2), 67.6 (C-6), 74–78 (C-3,4,5), 101.1 (C-1) and 178.1 ($COCH_3$). $[\alpha]_D^{27}$ −25.2° (*c* 1.0, HCOOH) for bromodeoxychitin of DS 0.85. Bromodeoxychitin of DS 0.92 (*N*-deacetylation, 5.7%), Anal. Calcd. for $(C_8H_{12}Br_{0.92}NO_4)_{0.943} \cdot (C_8H_{13}NO_5)_{0.943} \cdot (C_6H_{10}Br_{0.92}NO_3)_{0.057} \cdot (C_6H_{11}NO_4)_{0.057} \cdot (H_2O)_{0.55}$: C, 35.26; H, 4.90; N, 5.21; Br, 27.35. Found: C, 35.09; H, 5.00; N, 5.23; Br, 27.69.

Measurements.—^{13}C NMR spectra were recorded on a JEOL JNM-FX90Q spectrometer (JEOL, Ltd.) at 40°C and 22.53 MHz. For the measurement, a mixture of 30 mg of bromodeoxychitin and 200 μL of trifluoroacetic anhydride in 200 μL of CH_2Cl_2 was heated for 20 min at 110°C in a Pierce Reacti-Vial, and dried at 60°C under N_2. After three cycles of this procedure, 600 μL of trifluoroacetic acid-*d* and 40 μL of Me_4Si

(internal reference) were added into the vial under N_2. This solution was then transferred to an NMR tube with a liquid-handling pipette under N_2.

For WAXD measurements, samples were compressed into discs (0.45 mm thick and 10 mm in diameter) under 350 kg/cm^2. The measurements were performed on a Rigaku Rota Flex RU-200 equipped with a scintillation counter at 50 kV and 180 mA. Nickel-filtered CuK α radiation was used. The incorporated data were corrected for air scattering, polarization and interference factors. IR spectra were recorded on a JASCO FT/IR-3 spectrophotometer (JASCO, Ltd.) in KBr discs.

For GLC and GLC-MS measurements, chitin samples (5 mg) were dissolved in 3 mL of 35% HCl, degassed, and kept for 6 h at 80°C in Pierce hydrolysis-tubes. Each solution was transferred to a flask after hydrolysis and evaporated to dryness. The hydrogen chloride was removed from the solid by five cycles of dissolution in 40 mL of water and evaporation. The saccharides in the hydrolyzate were converted into *N,O*-TFA derivatives in a Pierce Reacti-Vial with 200 μL of trifluoroacetic anhydride in 200 μL of CH_2Cl_2 for 10 min at 110°C and dried under a stream of N_2. After two cycles of this procedure, CH_2Cl_2 (600 mL) was added into the vial under N_2. Apparatus and operation conditions for GLC and GLC-MS analyses were the same as those reported previously [4]. A glass column (3 m × 3 mm, i.d.) packed with 3% Silicone SE-30 on Gas Chrom Q (100–120 mesh) was used.

For the GPC measurements, a mixture of 4 mg of bromodeoxychitin, 200 μL of trifluoroacetic anhydride and 200 μL of CH_2Cl_2 was heated for 20 min at 110°C in a Pierce Reacti-Vial, and dried at 60°C under N_2. After two cycles of this procedure, 0.08 g/dL LiBr–DMF solution (4 mL) was added under N_2. Molecular-weight distributions of bromodeoxychitins after trifluoroacetylation were determined based on polystyrene standards with a Shodex DS-3 gel permeation chromatograph (Showa Denko Co.) on Shodex GPC KD-806M (x 2), KD-802 and KD-800P columns connected with RID-6A RI detectors (Shimadzu Corp.) and mini DAWN optical scattering detectors (Wyatt Technology Co.) with 0.08 g/dL LiBr–DMF as the eluent. Molecular-weight distribution curves were converted into DP distribution curves with use of the DS values of the samples. Peak areas were normalized for all samples. Solution viscosities were measured in 10 g/dL LiBr–DMA at 40°C.

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