

## Studies on Chitin. II. Preparation of Benzyl and Benzoylchitins

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(Received September 22, 1978)

**ABSTRACT:** Chitin was converted into benzyl and also benzoylchitins. Benzylchitins up to 0.8 benzylated were prepared by the reaction of alkali chitin with benzyl chloride in dimethyl sulfoxide. The solubility of benzylchitins in organic solvents was not so good, because of the low degree of benzylation. Various benzoylated chitins up to 1.8 mol benzoylated per residue were prepared by the benzylation of chitin in a benzoyl chloride-methanesulfonic acid mixture at 0°C. Benzoylchitins were soluble in many organic solvents, *e.g.*, dimethyl sulfoxide, dimethylformamide and benzyl alcohol, in addition to the acidic solvents such as formic acid. Infrared absorption spectra for benzyl and benzoylchitins were also investigated.

**KEY WORDS** Chitin / Benzylchitin / Benzoylchitin / Methanesulfonic Acid / Benzyl Chloride / Benzoyl Chloride / Solubility / Infrared Absorption Spectra /

Chitin, a polymer consisting of *N*-acetylglucosamine units, occurs very widely in nature and constitutes an essential supporting structure for several living organisms including fungi, algae, annelids, mollusks, and arthropods. Chitin exists in three different polymorphic forms, depicted as  $\alpha$ -chitin,  $\beta$ -chitin, and  $\gamma$ -chitin, depending upon the crystalline structure.<sup>1,2</sup>  $\alpha$ -Chitin is the most abundant and most stable natural polymorphic form, but during the process of isolation it is converted into the other forms.<sup>3,4</sup>

Chitin and chitin derivatives have been reported to have some useful medical applications. For example, chitin administration to animals attacked by certain bacteria and fungi has proved very useful as a highly effective antigen.<sup>5</sup> Chitin has been found suitable as a biodegradable pharmaceutical carrier,<sup>6</sup> a blood anti-coagulant,<sup>7</sup> a wound-healing accelerator,<sup>8</sup> and as a highly selective medium for the isolation of actinomycetes.<sup>9</sup> Chitosan membranes have also been proposed as artificial organ membranes because of their suitable permeability properties and high tensile strength.<sup>10</sup> Despite these medical applications, chitin

as a natural resource is still underutilized in comparison with other polysaccharides such as cellulose and starch.

Although chitin is structurally similar to cellulose, it has poor solubility and is more resistant toward chemical reagents because of the strong micelle structure of the acetamide groups. The poor solubility of chitin and chitin derivatives in common solvents has been the main drawback in its utilization. For example, cellulose acetate, which is soluble in many organic solvents has many practical uses as fibers, films, paints, and plastics but chitin acetate is practically insoluble in all solvents except acids, which tend to decompose it on standing.<sup>11</sup> Chitosan, which is deacetylated chitin, has a free amino group which promotes its solubility in some solvents, particularly those which are acidic in nature; the amino group also enhances its reactivity toward chemical reagents. Hence many derivatives of chitosan have been prepared and the practical uses of chitosan far outnumber those of chitin.

Chemically modified chitin derivatives with good solubility in organic solvents are highly desirable in order to promote the usefulness of chitin and widen the scope of its industrial applications. It is known that hydrophobic groups have affinity for organic solvents; therefore, the introduction of hydrophobic

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groups such as the benzyl or benzoyl group into the chitin molecule should enhance its solubility in common organic solvents. The preparation of such soluble chitin derivatives constituted the main aim of the investigation reported in this paper.

## EXPERIMENTAL

The elemental analyses for chitin, benzylchitin, and benzoylchitin were carried out by means of a Yanagimoto CHN Corder, Model MT-2. Thermal analyses were carried out with a Meiho Thermo Analyzer MP-2. The infrared absorption spectra were measured with a Hitachi Grating Infrared Spectrophotometer, EPI-G-2, by the KBr pellet method.

### Chitin

Powdered chitin of crab shell (Alaska King Crab) was obtained from Nippon Suisan Co. Ltd. Anal. Found: C, 44.96; H, 6.50; N, 6.57%. Calcd for  $C_8H_{13}NO_5 \cdot 1/2 H_2O$ : C, 45.28; H, 6.60; N, 6.60%.

### Preparation of Alkali Chitin

(a) Alkali chitin was prepared according to the previous paper.<sup>11</sup> (b) Alkali chitin was prepared by adding 1.77 ml of 40% KOH (21 mmol) to 1.49 g of chitin (7 mmol) suspended in 10 ml of DMSO; the mixture was stirred for 3–4 h at 10–12°C and kept for 10 h at 4°C. The alkali chitin was filtered off and washed with dry acetone to remove water. (c) Alkali chitin was prepared by the hydrolysis of diacetylchitin prepared according to the procedure recently reported by Nishi, *et al.*<sup>11</sup> Two grams of diacetylchitin (7 mmol) suspended in 10 ml of DMSO were hydrolyzed by stirring with 2.36 ml of 40% KOH (28 mmol) at 10–12°C for 3 h and kept in the refrigerator for another 12 h at 4°C. The alkali chitin was filtered off and washed with acetone to remove water.

Optimum conditions for the quantity of alkali required to produce alkali chitin which can be benzylated into benzylchitin were studied by varying the amount of potassium hydroxide added, as shown in Table I.

### Benzoylation of Alkali Chitin

The alkali chitin, from either (a), (b), or (c) as described above, suspended in DMSO (10 ml) was partially benzylated by adding 8.1 ml of benzyl

chloride (70 mmol) and stirring initially at 0–5°C for 1 h, during which the initially alkaline reaction medium became neutral. The mixture was stirred for another 20 h at room temperature, after which the slightly viscous mixture was still neutral. Ice-water was added to precipitate the product. The cold water was removed by decantation and another fresh portion of ice-water was added. The process was repeated until the initially semi-solid product solidified. The solid product was boiled in water for a few minutes before filtering the product, which was washed with water, ethanol, and ether to remove the last traces of unreacted benzyl chloride. The product was dried *in vacuo*.

Optimum conditions for the benzoylation reaction were determined by varying the quantity of benzyl chloride added, as shown in Table II.

### Benzoylation of Chitin

A 2.12 g (10 mmol) amount of chitin powder ( $\beta$ -form) was added to methanesulfonic acid (15 ml) with stirring at 0°C. 11.7 ml (100 mmol) of benzoyl chloride were added to the gel of chitin in methanesulfonic acid. The mixture was stirred for 3 h at 0°C and kept at –20°C for 18 h. The product, precipitated by the addition of ice-water, was filtered, washed with ice-water, and then was resuspended in ice-water. The suspension of the product, which was acidic to litmus, was neutralized by the cautious addition of dilute aqueous ammonia to pH 7.0, and boiled for a few minutes in water to dissolve any mineral salt present. The hot mixture was filtered and washed with distilled water. The dried product was further purified by suspending in ether; it was filtered and washed with ether to remove the last traces of benzoyl chloride. Then it was resuspended in ethanol, filtered, and washed with a little quantity of ethanol. The product was dried *in vacuo*.

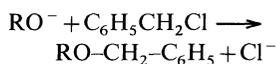
Products with different degree of benzoylation were achieved by varying the quantity of benzoyl chloride added, as shown in Table III, or by varying the period of stirring the mixture at 0°C before storage at –20°C, as shown in Table IV.

## RESULTS AND DISCUSSION

### Preparation of Benzylchitin from Alkali Chitin

Treatment of alkali chitin in aqueous KOH or NaOH with benzyl chloride did not produce the desired benzylchitin. Two factors can be responsible

for the lack of benzylation reaction. Firstly, the aqueous alkaline potassium or sodium hydroxide could promote the hydrolysis of benzyl chloride and secondly the presence of water could inhibit the reaction, probably by hydrolysing the alkoxide. Inhibition of the benzylation of sodium cellulose by water has previously been reported by Wolfrom, *et al.*<sup>12</sup> When alkali chitin is treated with benzyl chloride in the absence of water, only 0.4 mol of hydroxyl groups per *N*-acetyl glucosamine residue can be benzylated reproducibly. A higher degree of benzylation could not be obtained even on treatment with excess benzyl chloride. The low degree of benzylation is probably due to the low alkoxide content of the alkali chitin. The benzylation reaction can be considered as a typical nucleophilic displacement reaction.



In the case of cellulose, Wolfrom, *et al.*, concluded that benzylation of alkali cellulose with benzyl chloride is a difficult reaction because of the topochemical and heterogeneous nature of the reaction. Despite employing very high temperatures and a reaction period of 48 h, only 0.92–1.0 hydroxyl units per  $\text{C}_6$  unit could be benzylated. Because of the susceptibility of the acetamide groups in chitin, high reaction temperatures can not be employed.

It has been suggested that diacetylchitin has better solubility in formic acid because the strong micelle structure characteristic of chitin has been partially reduced in the ester; hence alkali chitin was prepared by the deacetylation of diacetylchitin. The alkali chitin from diacetylchitin has a better swelling property in DMSO than the alkali chitin prepared directly from chitin. This alkali chitin, derived from

the ester, gave a product on benzylation in which 0.8 mol of OH groups per *N*-acetyl glucosamine unit were benzylated.

The results in Table II show that the highest degree of benzylation occurs when the molar ratio of benzyl chloride to *N*-acetyl glucosamine residue is 10:1. The results in Table I show that potassium hydroxide inhibited benzylation reaction. At least two moles of potassium hydroxide per mole of acetyl residue in diacetylchitin are necessary to effect complete hydrolysis of the acetate and convert the resulting alcohol to the alkoxide.

The infrared absorption spectrum of partially benzylated chitin shows the characteristic absorptions due to the aromatic ring at 1960, 1880, 845, 820, 760, and 705  $\text{cm}^{-1}$  as shown in Figure 1. Only the primary alcohol group seems to be reacted in the case of benzylation, because the benzylchitin was obtained through alkali chitin, although the evidence for the substituted position was not clearly given from the IR spectra.

#### Preparation of Benzoylchitin

Recently we reported the acetylation of chitin using acetic anhydride in methanesulfonic acid. Attempts to prepare the benzoyl analog of chitin by a similar procedure were unsuccessful because of the decomposition of benzoic anhydride in methanesulfonic acid. In another procedure, benzoylation was carried out using benzoyl chloride as the benzoylating reagent. Ordinarily, benzoylations of amino and hydroxyl functional groups proceed smoothly with benzoyl chloride under basic conditions. By applying such conditions, cellulose has been reported to be successfully benzoylated, but under such conditions benzoylation of chitin was not achieved. Successful benzoylation of chitin was achieved only when chitin

**Table I.** The relationship between the degree of benzylation and potassium hydroxide added

Molar ratio <sup>a</sup>	Degree of <sup>b</sup> benzylation	Found, %			Calcd, %		
		C	H	N	C	H	N
4	0.75	56.75	6.62	4.87	56.88	6.83	5.01
8	0.3	50.42	6.40	5.38	50.71	6.61	5.86
20	0.1	47.16	6.59	5.89	47.24	6.61	6.33

<sup>a</sup> Equivalent moles of potassium hydroxide per *N*-acetylglucosamine residue in the presence of 10 equivalent moles of benzyl chloride.

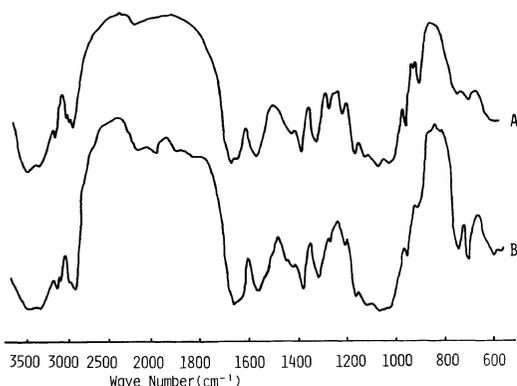
<sup>b</sup> Benzylated groups per *N*-acetylglucosamine residue.

**Table II.** The relationship between the degree of benzylation and benzyl chloride added

Molar ratio <sup>a</sup>	Degree of <sup>b</sup> benzylation	Found, %			Calcd, %		
		C	H	N	C	H	N
2	0.1	46.97	6.74	5.86	47.24	6.61	6.33
4	0.3	50.92	6.41	5.34	50.71	6.61	5.86
8	0.6	55.24	6.60	5.12	55.04	6.62	5.26
10	0.75	56.75	6.62	4.87	56.88	6.83	5.01
16	0.5	53.17	6.27	5.06	53.70	6.61	5.45

<sup>a</sup> Equivalent moles of benzyl chloride per *N*-acetylglucosamine residue in the presence of 4 equivalent moles of potassium hydroxide.

<sup>b</sup> The same as in Table I.

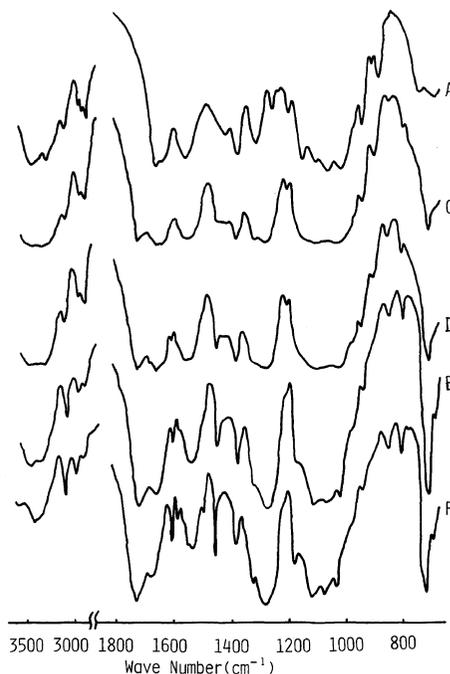


**Figure 1.** Infrared absorption spectra of chitin and benzylchitin: A, chitin; B, 0.75 benzylchitin.

was dissolved into a gel in methanesulfonic acid and treated with benzoyl chloride. Methanesulfonic acid was a suitable solvent as well as a catalyst for the homogeneous benzoylation reaction. The reaction temperature was set at 0°C to prevent the decomposition of chitin by methanesulfonic acid.

The results in Table III show that the optimum degree of benzoylation was achieved when the molar ratio of benzoyl chloride to the *N*-acetyl glucosamine residue was 10:1.

The results in Table IV show that the highest degree of benzoylation was attained by stirring the mixture of chitin and benzoyl chloride for 4.5 h at 0°C before keeping it in the freezer at -20°C overnight. Prolonging the time of stirring did not seem to affect the degree of benzoylation. Storage of the reaction mixtures overnight at 4°C or -20°C was found to give the same degree of benzoylation. However, overnight storage at -20°C was chosen, because the benzoylchitins prepared by the freezing



**Figure 2.** Infrared absorption spectra of chitin and benzoylchitins: A, chitin; C, 0.3 benzoylchitin; D, 0.6 benzoylchitin; E, 1.2 benzoylchitin; F, 1.8 benzoylchitin.

procedure have better solubility properties than the benzoylchitin stored at 4°C. These results agreed with the report that the freezing aids the weakening of the strong micelle structure, with consequent improvement of the solubility properties.<sup>11</sup>

The infrared absorption spectra of chitin and benzoylchitin with varying degree of benzoylation are shown in Figure 2. Pearson, *et al.*,<sup>3</sup> and Gardner and Blackwell<sup>4</sup> have demonstrated that natural

chitin exists in the  $\alpha$ -polymorphic form and during the process of isolation it is converted to the  $\beta$ -form. The main distinguishing feature of the IR spectra of the two forms is that the  $\alpha$ -form has a characteristic absorption at  $1660\text{ cm}^{-1}$ , whereas the  $\beta$ -form has two absorptions at  $1620$  and  $1660\text{ cm}^{-1}$ . Since the chitin used in this investigation has two absorptions at  $1620$  and  $1660\text{ cm}^{-1}$ , it is assumed to be in the  $\beta$ -form. The spectra in Figure 2 show that as the degree of benzylation increases there is a corresponding decrease in the absorptions at  $3480$  and  $3447\text{ cm}^{-1}$ , which are due to the hydroxyl groups, and new absorption frequencies appear at  $1730$  and  $1280\text{ cm}^{-1}$ , which are characteristic of the benzoyl ester. The absorptions characteristic of the benzene ring also appear at  $1980$ ,  $1920$ ,  $1610$ ,  $850$ ,  $810$ , and  $720\text{ cm}^{-1}$  with the absorptions at  $750$  and  $1620\text{ cm}^{-1}$ , which are characteristic of out of plane bending of the imino (NH) group and adsorbed water respectively, rapidly diminishing as the degree of benzylation increases. As shown in Figure 2,  $\text{C}_6\text{-OH}$  group (primary alcohol) seems to be sub-

stituted predominantly by benzoyl group, because the decrease of absorption at  $3480\text{ cm}^{-1}$  (primary OH) was followed by the decrease at  $3447\text{ cm}^{-1}$  (secondary OH).<sup>3,4</sup>

The results of elemental analysis, infrared absorption, and thermal analysis suggest that chitin contains  $0.5$  mol of water per *N*-acetylglucosamine residue. As in the case of monoacetylchitin, monobenzylchitin and monobenzoylchitin also binds  $0.5$  mol of water per *N*-acetylglucosamine unit, which was released at  $115^\circ\text{C}$  as reported previously.<sup>11</sup> The composition of the intermediates for benzylation and benzoylation agrees well with the monosubstituted chitin binding half a mole of water, and there is no bound water in the disubstituted chitin, as in the data reported for acetylchitin.<sup>11</sup>

#### Solubility Properties of Benzyl and Benzoylchitins

The solubility properties of the partially benzylated chitin are not significantly better than those of the previously reported diacetylchitin, presumably because of the low degree of benzylation, although the

**Table III.** The relationship between the degree of benzylation and benzoyl chloride added

Molar ratio <sup>a</sup>	Degree of <sup>b</sup> benzylation	Found, %			Calcd, %		
		C	H	N	C	H	N
2	0.3	49.52	6.31	5.69	49.84	6.25	5.76
4	0.6	53.15	5.98	5.05	53.35	5.98	5.10
8	1.2	58.29	5.18	4.14	58.43	5.58	4.16
10	1.7	61.03	5.07	3.73	61.42	5.35	3.60
20	1.6	60.72	5.14	3.94	60.88	5.39	3.70

<sup>a</sup> Equivalent moles of benzoyl chloride per *N*-acetylglucosamine residue, stirring at  $0^\circ\text{C}$  for 3 h and storage at  $-20^\circ\text{C}$ .

<sup>b</sup> Benzoylated groups per *N*-acetyl glucosamine residue.

**Table IV.** The relationship between the degree of benzylation and time

Time, h <sup>a</sup>	Degree of <sup>b</sup> benzylation	Found, %			Calcd, %		
		C	H	N	C	H	N
1	0.5	52.13	5.64	5.18	52.27	6.06	5.30
2	1.7	61.03	5.12	3.83	61.42	5.35	3.60
3	1.7	61.37	5.11	3.84	61.42	5.35	3.60
4.5	1.8	61.77	5.07	3.79	61.92	5.31	3.50

<sup>a</sup> Period of stirring at  $0^\circ\text{C}$  before storage at  $-20^\circ\text{C}$  with 10 molar equivalent of benzoyl chloride per *N*-acetylglucosamine residue.

<sup>b</sup> The same as in Table III.

solubility in formic acid was improved by the benzylation. The solubility properties of benzoylchitin are remarkable. It has very good solubility properties in common organic solvents. A higher degree of benzylation thus gave rise to better solubility. A 1.8 benzyolated chitin is easily soluble in benzyl alcohol and dimethyl sulfoxide, in addition to the acidic solvents such as formic acid or dichloroacetic acid, and forms a clear and viscous solution. It is also fairly soluble in dimethylformamide, hexamethylphosphoramide, dimethylacetamide, and some sorts of mixed solvent system, *e.g.*, mixtures of alkyl halides such as tetrachloroethane or dichloroethane and alcohols such as ethyl alcohol or butyl alcohol. The good solubility properties of benzoylchitin in organic solvents will enhance the possibility of its utilization.

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