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Preparation of Alcohol-Soluble Chitin Derivatives and Radical Induction by Photo-Irradiation

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ABSTRACT

A water-soluble 6-O-carboxymethyl (CM)-chitin was converted into a methanol-soluble one through an alkylation reaction by allyl bromide under the basic conditions. The allyl-CM-chitin (A-CM-chitin) produced was regenerated into a sparingly soluble film, when A-CM-chitin–methanol solution was cast on a glass plate and dried under light. The induction of the allyl radical was observed with both the ESR spectrum and 1,1-diphenyl-2-picrylhydrazyl (DPPH) titration after irradiation of visible light. The double bond introduced was also re-formed into a propynyl group through halogenation of the allyl group and the dehydrohalide reaction.

INTRODUCTION

Although chitin is known to be a sparingly soluble mucopolysaccharide owing to the rigid crystalline structure, a hydrophilic property has been

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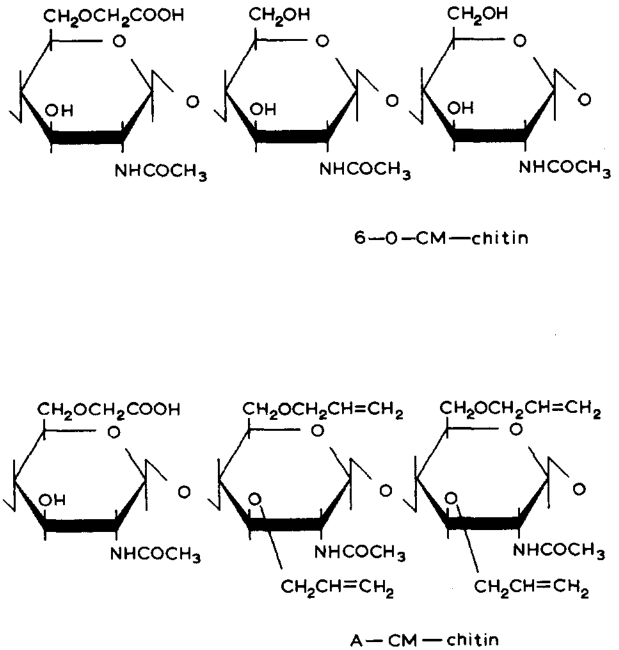
demonstrated for the chitin molecule by substitution to destroy the crystalline structure, such as alkylation reactions in which the C-6 hydroxyl group on GlcNAc residue was attacked predominantly (Tokura *et al.*, 1983*a*). A 6-*O*-carboxymethyl-chitin (6-*O*-CM-chitin) has been prepared by the direct alkylation reaction by applying monochloroacetic acid to alkali–chitin under basic conditions. The biodegradability of chitin was enhanced remarkably by carboxymethylation (Tokura *et al.*, 1983*b*). Since the destruction of the crystalline structure to increase hydrophilicity was achieved by the alkylation of chitin even at a low degree of substitution, subsequent chemical modification was expected to proceed smoothly, including substitution of the C-3 hydroxyl group of the GlcNAc residue (Nishimura *et al.*, 1986).

In the present study, 6-*O*-CM-chitin was allylated by allyl-bromide under basic conditions (Scheme 1). The resulting allyl-CM-chitins (A-CM-chitins) were found to be soluble in alcohols, especially in methanol. But A-CM-chitins were regenerated into a sparingly soluble chitin derivative by drying under the light. Since IR spectra of the induced allyl group indicated the conversion of the double bond to carbonyl derivatives under the irradiation of a mercury lamp in air, the ESR spectrum was obtained and chemical titration was performed to investigate the stability of the induced radicals.



Scheme 1. Chemical structures of chitin, 6-*O*-carboxymethyl (CM)-chitin, and allylated CM-chitin (A-CM-chitin).

EXPERIMENTAL

Reagents

Reagents of chemical grade were purchased from Wako Pure Chemical Co. Ltd and Tokyo Kasei Kogyo Co. Ltd and used without further purification.

CM-chitins

Carboxymethyl-chitins were prepared from chitin powder (60–120 mesh) of Queen Crab shells and monochloroacetic acid under basic conditions according to the method reported previously (Tokura *et al.*, 1983*c*). The degree of substitution was controlled by the concentration of sodium hydroxide in the preparation of alkali–chitin. Variously substituted CM-chitins were obtained as dried powder after successive washing by water, acetone, and ethanol in the case of a water-insoluble CM-chitin or after successive washing of the acetone precipitate with ethanol and ether in the case of a water-soluble CM-chitin.

A-CM-chitin

Carboxymethyl-chitin (Na salt) (10 g) was suspended or dissolved in 35 ml of 40% (w/w) aqueous sodium hydroxide solution (containing 0·02% SDS) at 4°C. It was then frozen at − 20°C overnight after standing for 1 h at 4°C. The frozen block was thawed in 10 ml of 2-propanol at room temperature, and 50 ml of allyl bromide (more than 10 equivalent moles) were added and it was allowed to stand for 24 h at room temperature. The reaction mixture was then heated to 40°C and allowed to stand for 48 h after the addition of a trace of iodide. A-CM-chitin was extracted with ethanol and reprecipitated by being poured into distilled water. The precipitate was collected by centrifugation and resuspended in distilled water repeatedly to remove free salts. The resulting A-CM-chitin was stored in the dark and wet to prevent the formation of cross-links and the regeneration of hydrogen bonds.

Degree of substitution

The degree of carboxymethylation was estimated by potentiometric titration with 0·1 M aqueous HCl solution in 0·1 M NaCl solution. The degree of allylation was calculated from the elemental analysis and from the flask-combustion method to detect the attached bromine group (Schoniger, 1955).

^{13}C-CP/MAS-NMR measurement

^{13}C-CP/MAS-NMR spectra of CM-, A-CM-, and A-chitins were obtained at 100 MHz by accumulation of 512 with a Brucker MSL-400 NMR spectrometer by using TMS as a standard. SSB was depressed by TOSS in the solid-state NMR measurements.

FT-IR spectra

A COO-Na type of A-CM-chitin-methanol solution was cast on a glass plate and dried at room temperature to prepare a thin film. The A-CM-chitin film was then treated with 1 M HCl in aqueous methanol (50% v/v) to convert to the COOH type of A-CM-chitin film. A-CM-chitin film was sealed in a quartz tube with air or in a nitrogen stream and the tube was then set under high-pressure mercury-lamp irradiation for various time intervals. Infrared spectra of A-CM-chitin films were measured in the sealed quartz tube by a Nicolet 5DXB Fourier-transform IR spectrometer with 4 cm^{-1} of resolution at 20°C. The base line was also corrected to set the absorbance to zero at 2200 cm^{-1}.

Detection of radicals

Photo-irradiated A-CM-chitin powder was suspended in 0·001% 1,1-diphenyl-2-picrylhydrazyl (DPPH) benzene solution to detect induced radicals after standing for 1 h at room temperature. The optical density of supernatant was measured at 530 nm by the Hitachi automatic spectrometer U-3200 (Henglein, 1955). Photo-induced radicals were also investigated by using a JEOL X-band ESR spectrometer (JES-FE-1XG) with a 9·2 GHz microwave power of 1 mW, 100-kHz field-modulation width of 2 G, and 4 min of sweep time at various temperatures.

RESULTS AND DISCUSSION

Allylation and solubility

The solubility of A-CM-chitin towards methanol has been shown to depend on the degree of previous alkylation as shown in Fig. 1 and reached a maximum at around 0·8 carboxymethylation in which 80% of C-6 hydroxyl groups of GlcNAc residue were substituted. The methanol-solubility was found only for the COO-Na type of A-CM-chitins and not for the COO-H type. The previous alkylation of chitin, such as sec-butylation at low degrees, also seems to be effective

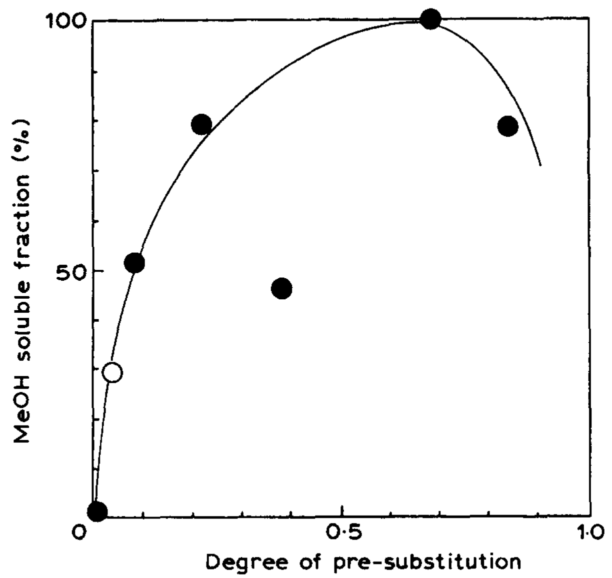


Fig. 1. Dependence of methanol-soluble fraction after allylation on the degree of pre-alkylation of chitin: —●—, allylation of 6-*O*-CM-chitins; —○—, allylation of sec-butyl-chitin.

TABLE 1
Degree of Allylation

	By elemental analysis	*By bromination*
A-0·08 CM-chitin	1·97	1·41
A-0·22 CM-chitin	2·00	1·31
A-0·38 CM-chitin	2·02	1·43
A-0·68 CM-chitin	2·40	1·45
A-0·84 CM-chitin	1·01	0·75
A-chitin	2·13	0·75
A-sec-butyl-chitin	2·00	0·75

for obtaining alcohol-soluble derivatives. The direct allylation of chitin did not give any methanol-soluble fractions owing to the poor substitution. Since the methanol-solubility of chitin and poorly alkylated chitin was not improved by a single allylation reaction, two-step allylation is required to prepare the methanol-soluble derivative in spite of the slight deacetylation of the chitin molecule. The degree of allylation was almost independent of the degree of carboxymethylation except for 0·8 CM-chitin, as shown in Table 1. Although there are big differences in both analytical values, the bromination value would correspond to the reactive allyl group, and the higher allyl values from elemental analysis than those from bromination suggest graft copolymerization. The average degree of graft copolymerization was estimated to be 5 to 7. The number of grafted chains is assumed to depend on the surface area of chitin or alkyl chitin powder from the closeness of their values because their particle sizes were almost identical. The low allylation value for 0·84 CM-chitin seems to be caused by steric hindrance with pre-induced carboxymethyl groups.

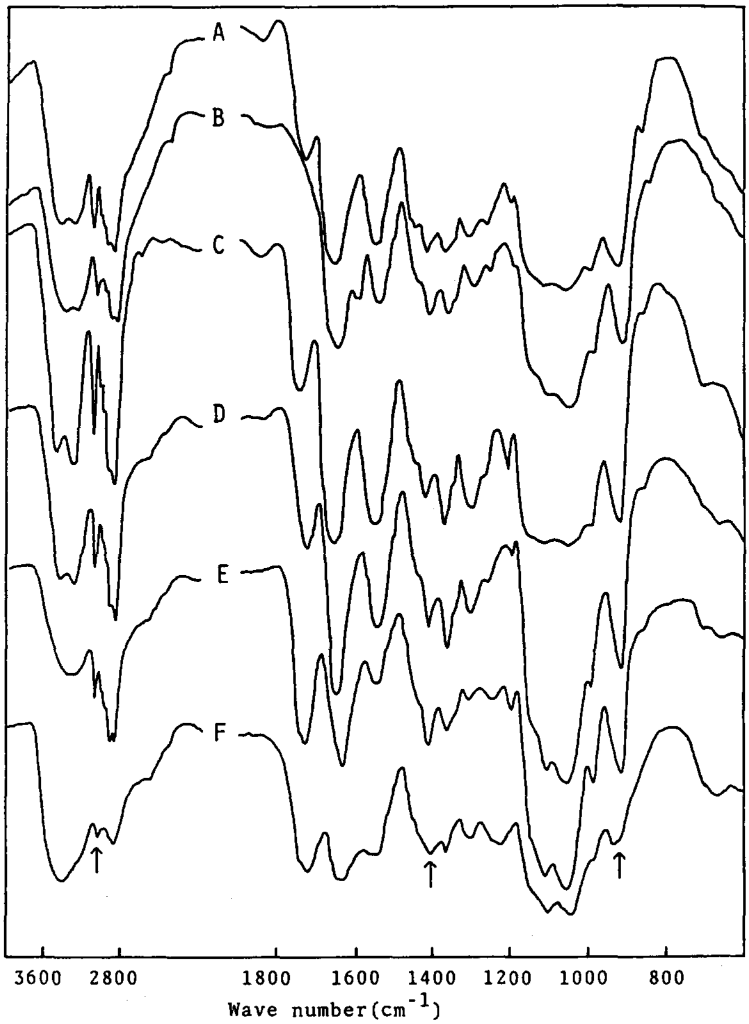


Fig. 2. Infrared spectra of various A-CM-chitins: A = A-0·08 CM-chitin; B = A-0·08 CM-chitin-Na salt; C = A-0·22 CM-chitin; D = A-0·38 CM-chitin; E = A-0·68 CM-chitin; F = A-0·84 CM-chitin.

Infrared spectra of methanol-soluble fractions are shown in Fig. 2. There are specific absorptions for the induced allyl group at 3080 and 2980 cm^{-1} owing to stretching of CH, at 3020 cm^{-1} owing to CH stretching, at 1420 cm^{-1} owing to in-plane deformation of CH, at 920 owing to out-of-plane deformation of the CH of the vinyl group, and also

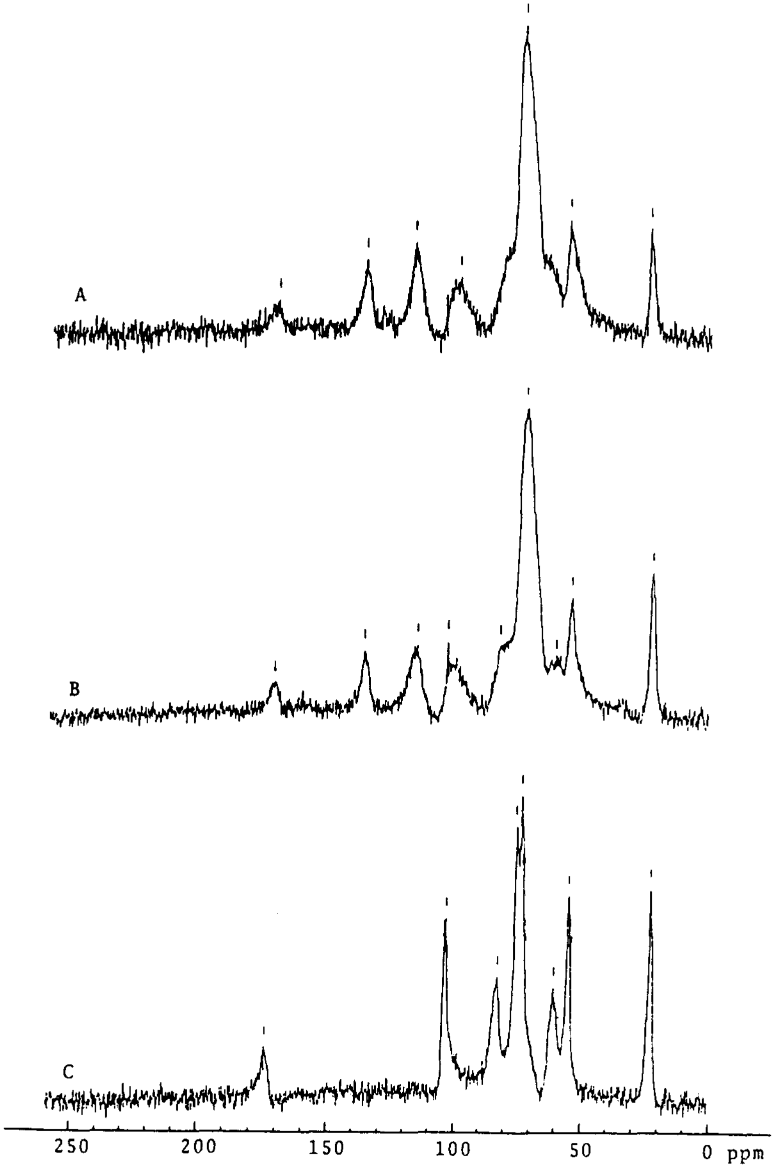


Fig. 3. ^{13}C-CP/MAS-NMR spectra (100 MHz) of chitin derivatives in the solid state: A= A-0·38 CM-chitin; B = A-chitin; C = 0·38 CM-chitin.

at 2930 and 2870 cm^{-1} owing to stretching of the CH of the allyl group. A slight transition of the double bond was suggested during the allylation reaction, because few peaks are observed between 730 and 675 cm^{-1} and around 965 cm^{-1}.

A ^{13}C-CP/MAS-NMR study was applied to chitin derivatives in the solid state as shown in Fig. 3 and Table 2. There are two peaks between 110 and 130 ppm owing to the induction of the double bond. Although there is little difference between the chemical shift of CM-chitin and that of A-chitin, the chemical shift due to the carbonyl of the acetamide group is shifted to a higher magnetic field on A-chitin. Since this phenomenon is also observed on the A-CM-chitin, several types of interaction are suggested between the carbonyl and ethylene groups of A-CM-chitins. As chemical shifts of the carbons of C-1 and C-4 of the GlcNAc residue were shifted to a higher magnetic field after allylation, it is suggested that glycoside linkage is distorted by the induced allyl groups.

Stability of the allyl group

The content of allyl groups tended to reduce gradually when A-CM-chitin film was irradiated under the mercury lamp in air as shown in Fig. 4. The photo-induced radicals were found to be more stable in a nitrogen atmosphere than in air as shown in Fig. 5, which was obtained when A-CM-chitin was stood for 30 min in the dark. Faster depression of several peaks owing to the hydroxyl and allyl groups was observed

TABLE 2

Comparison of ^{13}C-NMR Chemical Shifts of Chitin Derivatives in the Solid State[a]

	0·38 CM-chitin	*A-chitin*	*A-0·38 CM-chitin*
C-1	103·09	103·36	98·90
C-2	54·97	54·95	55·52
C-3	73·25	72·87[b]	72·78[b]
C-4	82·69	82·68	81·06
C-5	75·49	72·87[b]	72·78[b]
C-6	60·87	61·06	63·58
CH_3	22·66	23·19	23·57
C=O	174·28	171·49	170·70
$=CH_2$	—	115·31	116·80
=CH	—	136·17	136·16
$-CH_2$	—	72·87[b]	72·78[b]

[a] ppm from TMS.
[b] C-3, C-5, and $-CH_2$ signals overlap.

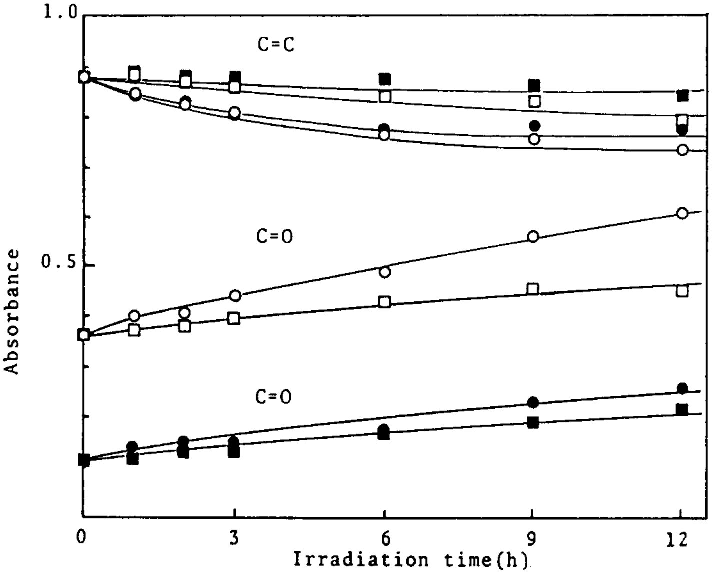


Fig. 4. Effect of photo-irradiation on A-CM-chitin films: —○—, A-CM-chitin H type; —●—, sodium salt in air; —□—, A-CM-chitin H type; —■—, sodium salt in N_2.

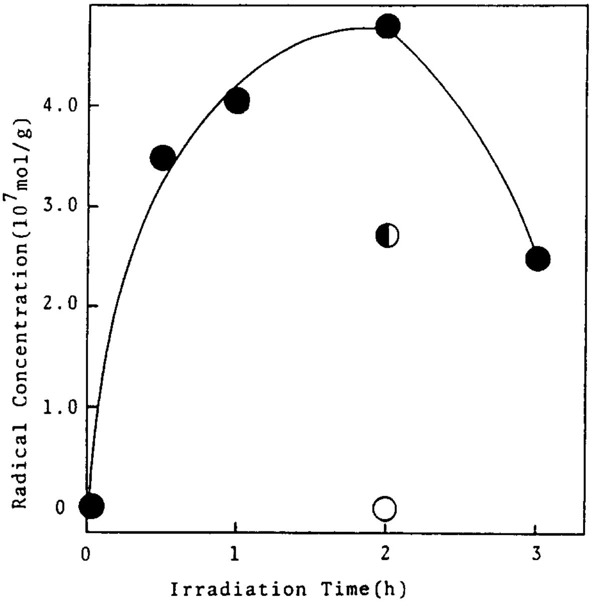


Fig. 5. Progress with time of radical production by irradiation and stability of radicals in air and in a nitrogen stream: —●—, radicals produced by irradiation; —○—, radical level after standing for 30 min in the dark and in air; —◐—, radical level after standing for 30 min in the dark and in a nitrogen stream.

through irradiation by visible light in air than in a nitrogen stream. This fact would suggest oxidative cleavage of the double bond by irradiation in air. Visible light was shown to be sufficient for the induction of radicals on the allyl group, because an almost identical production of radicals was observed by irradiation through glass and quartz plates.

ESR spectra

ESR spectra of chitin derivatives (A-CM-chitin-Na, A-CM-chitin-H, A-chitin, and A-sec-butyl-chitin) are shown in Fig. 6. Only A-CM-chitin-

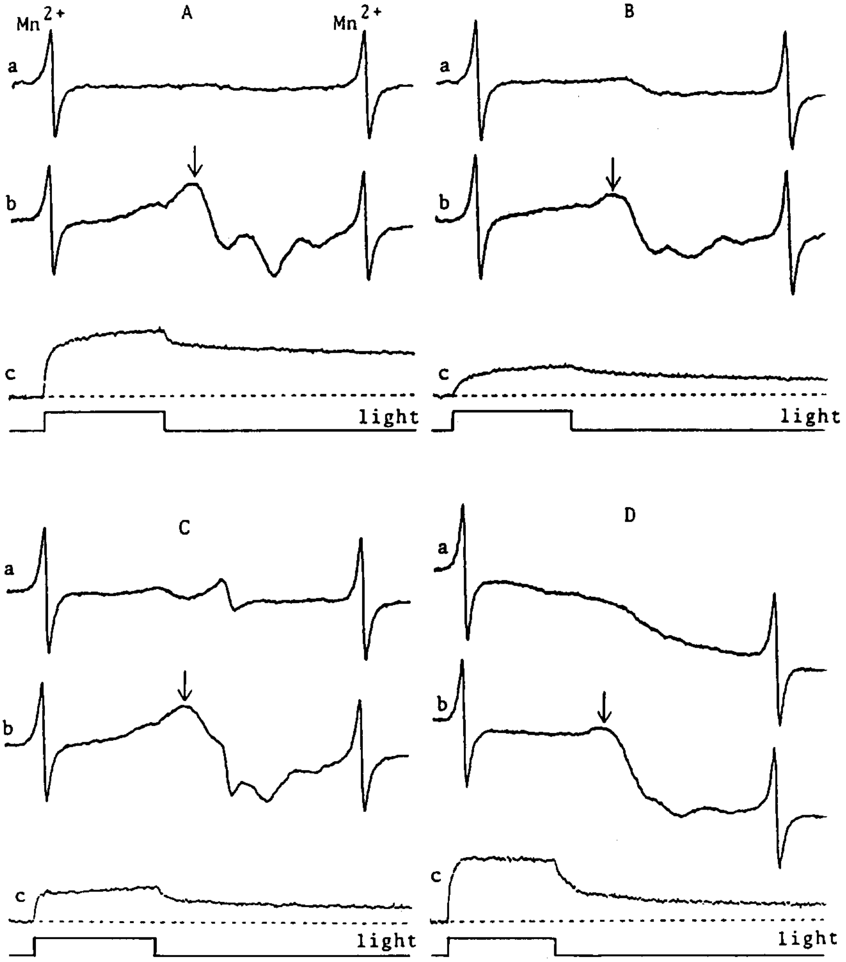


Fig. 6. ESR spectra of chitin derivatives before (a) and under irradiation (b) with monitored radical intensity (c). Radical intensity was monitored at the peak indicated by an arrow: A = A-CM-chitin-Na; B = A-chitin; C = A-CM-chitin-H type; and D = A-sec-butyl-chitin.

Na of these derivatives produced long-life radicals by irradiation, because the radical intensity (C in Fig. 6) of A-CM-chitin-Na was reduced very slowly and was still sharp even after 20 min at room temperature in the dark. Since several peaks due to photogenerated radicals of A-CM-chitin-Na were observed after standing for 20 min in the dark at −140°C as shown in Fig. 7, allyl groups may be buried in several kinds of domain.

Various interactions between allyl and other functional groups are now under investigation.

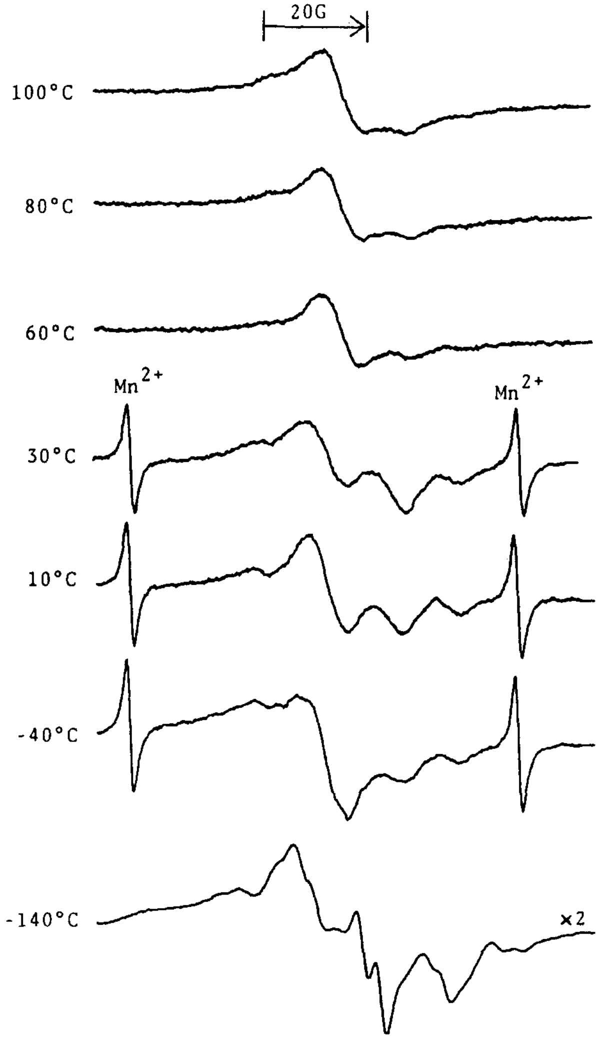


Fig. 7. Temperature-dependence of ESR spectra of photogenerated radicals of A-0·38 CM-chitin-Na.

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