

Characterization of epoxidized natural rubber by 2D NMR spectroscopy

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Abstract

Assignment of signals in aliphatic region of ¹H NMR spectrum for epoxidized natural rubber was carried out through NMR spectroscopy. The epoxidized natural rubber was prepared by epoxidation of purified natural rubber with peracetic acid in latex stage followed by degradation with propanal and ammonium persulfate. The resulting liquid epoxidized natural rubber was characterized through 1D- and 2D-NMR spectroscopy. The unknown signals in the aliphatic region of the ¹H NMR spectrum were assigned through ¹³C NMR and two-dimensional heteronuclear shift correlation (HETCOR) measurement. The assignments were proved by two-dimensional inverse detected heteronuclear long-range shift correlation (HMBC) and two-dimensional homonuclear shift correlation (COSY) measurements, and they were supported with epoxidized squalene as a model compound through NMR spectroscopy.

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1. Introduction

High-resolution nuclear magnetic resonance (NMR) spectroscopy has made possible to characterize primary structure of polymers that has been observed ever, due to the increase in magnetic field. Especially, solution state NMR spectroscopy is recognized to be indispensable for the characterization, in which the signals are systematically assigned with regard to chemical shift, coupling constant and intensity ratio as well as spin coupling between homo- and hetero-nuclei. For example, 2D NMR measurement such as HETCOR, HMBC and COSY enables to assign the complicated signals, which were difficult to assign with 1D NMR measurement.

Signals in aliphatic region of ¹H NMR spectrum for epoxidized natural rubber were independently reported by Bhattacharjee et al. [1] and Thames and Gupta [2]. However, in the previous works, several signals in the aliphatic region were

not assigned because of low magnetic field of spectrometer and formation of three-dimensional network during epoxidation. In fact, the assignment of the signal at 1.6 ppm, performed by Bhattacharjee and Thames, was inconsistent with each other. Hence, the signals in the aliphatic region have not been useful for estimation of epoxy group content of the sample.

The epoxy group content has been estimated from intensity ratio of the signals at 2.7 and 5.1 ppm, which were assigned to methine proton of epoxy group and unsaturated methine proton of isoprene unit of epoxidized natural rubber, respectively. However, in some cases, the signal at 5.1 ppm cannot be used for the estimation of epoxy group content, because the unsaturated methine proton of the isoprene unit may cause side reactions during epoxidation. Thus, for the sake of the estimation of epoxy group content, it is necessary to use the signals in the aliphatic region. From this point of view, the signals in the aliphatic region have to be correctly assigned.

Epoxidized natural rubber has been prepared in solution or latex stage by reacting natural rubber with peroxide. During epoxidation of the rubber, gel fractions are formed due to

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formations of both physical crosslinking point caused by proteins present in natural rubber and chemical crosslinking point caused by ring opening reaction of epoxy group [3–5]. Hence, for the assignment of the ^1H NMR spectrum, it is necessary to remove proteins from natural rubber and to decompose the gel fraction. In the previous work, we established rapid and efficient purification procedure of natural rubber with urea [6,7]. Resulting hyper-deproteinized natural rubber (DPNR) was subjected to epoxidation followed by degradation to prepare liquid epoxidized natural rubber (LEDPNR), which has high molecular mobility [8,9]. Since the LEDPNR is linear polymer with narrow molecular weight distribution, it will be precisely characterized through solution state NMR spectroscopy.

In the present study, assignment of the signals in the aliphatic region of ^1H NMR spectrum for LEDPNR was carried out by 1D- and 2D-NMR measurements to establish the new estimation method for epoxy group content.

2. Experimental

Natural rubber latex used in this study was commercial high ammonia natural rubber (HA-NR) latex. Deproteinization of natural rubber was made by incubation of the latex with 0.1% w/w urea (Nacalai Tesque Inc., 99.5%) in the presence of 1% w/w sodium dodecyl sulfate (Kishida Reagents Chemicals Co. Ltd., 99%) at 303 K for 1 h followed by centrifugation at 10^4g . Resulting deproteinized natural rubber latex, pre-cooled at 283 K, was epoxidized in the latex stage with fresh peracetic acid (33% v/v concentration) for 3 h. After completion of the reaction, pH of the solution was adjusted to 9–10 with ammonia solution (Nacalai Tesque Inc., 28%). Degradation of the epoxidized DPNR was carried out by incubation of the epoxidized DPNR latex with ammonium persulfate (Nacalai Tesque Inc., 99.5%) and propanal (Nacalai Tesque Inc., 99.5%) at 338 K for 10 h to prepare LEDPNR. The latex was coagulated with methanol (Nacalai Tesque Inc., 99%) followed by purification with toluene (Nacalai Tesque Inc., 99.5%) and methanol, and dried up at 303 K for a week under reduced pressure.

Squalene (Tokyo kasei Inc., 96.0%), pre-cooled at 283 K, was epoxidized with fresh peracetic acid (33% v/v concentration) for 3 h. After completion of the reaction, resulting partially epoxidized squalene was washed with water to adjust pH to 7.

Apparent molecular weights and molecular weight distribution of polymers were determined by GPC system of Tosoh Ltd. with a computer control dual pump, a RI-8012 differential refractive index detector, a UV-8011 ultraviolet spectroscopic detector, and a series of three G4000H_{HR} columns (bead size is 5 μm , pore size is 10^4 \AA) with 300 mm length and 7.8 mm i.d. each. THF was used as an eluent, and the flow rate was 0.5 ml/min, at room temperature. Standard polystyrenes were used for calibration.

NMR measurements were carried out using a JEOL ECA-400 NMR spectrometer operating at 399.65 and 100.4 MHz for ^1H and ^{13}C , respectively. The polymer was dissolved

into chloroform-*d* without tetramethylsilane (TMS). Chemical shifts were referred to chloroform in chloroform-*d*. ^1H -, ^{13}C NMR and distortionless enhancement by polarization transfer (DEPT) measurements were carried out at 323 K at the pulse repetition time of 7 s and 5 s, respectively. Two-dimensional COSY, HETCOR and HMBC measurements were made to collect two-dimensional hyper complex data. After weighing with shifted sine-bell function, the data were Fourier-transformed in the absolute value mode.

3. Results and discussion

3.1. Characterization of LEDPNR

Table 1 shows number average molecular weight, M_n , weight average molecular weight, M_w , polydispersity index, M_w/M_n , and gel content of DPNR and LEDPNR, respectively. Since the values of M_n , M_w and gel content of LEDPNR were significantly less than those of DPNR, characterization of this sample was carried out through solution state NMR spectroscopy.

Fig. 1 shows typical ^1H NMR spectra for DPNR and LEDPNR. As for DPNR, three signals characteristic of methyl, methylene and unsaturated methine proton of *cis*-1,4-isoprene unit appeared at 1.68, 2.05 and 5.1 ppm, respectively. In contrast, for LEDPNR, two signals characteristic of epoxy group appeared at 1.29 and 2.7 ppm, which were assigned to methyl and methine proton of resulting epoxy group, respectively, while unknown signals appeared at 1.55 and 2.15 ppm. The unknown signals were overlapping with the signals at 1.68 and 2.05 ppm. The epoxy group content of LEDPNR, X_{epoxy} , was estimated from the intensity ratio of the signals at 2.7 and 5.1 ppm, as in the following equation:

$$X_{\text{epoxy}} = \frac{I_{2.7}}{I_{2.7} + I_{5.1}} \times 100 \quad (1)$$

where I is intensity of the signals and subscript numbers represent chemical shift (ppm). The estimated epoxy group content of LEDPNR used in the present study was 33%.

To assign the unknown signals at 1.55 and 2.15 ppm, various combinations of triad sequences of epoxidized isoprene unit (E) and unepoxidized isoprene unit (C) were taken into account, according to Bradbury and Perera [5], as shown in Fig. 2. The carbon atoms of each middle unit in triad sequences were numbered to distinguish from each other. For example, the carbon atoms of middle unit in CCC triad sequence were described as CC^1C , CC^2C , CC^3C , CC^4C and CC^5C , in which the C represents *cis*-1,4-isoprene unit, the CCC is a triad sequence of the *cis*-1,4-isoprene units and the superscript indicates the numbering of carbon atoms of the middle unit as shown in Fig. 2.

Table 1
 M_n , M_w , M_w/M_n and gel content for DPNR and LEDPNR

	M_n (10^5)	M_w (10^5)	M_w/M_n	Gel content (%)
DPNR	2.1	17.8	8.5	60.9
LEDPNR	0.17	0.48	2.8	0.0

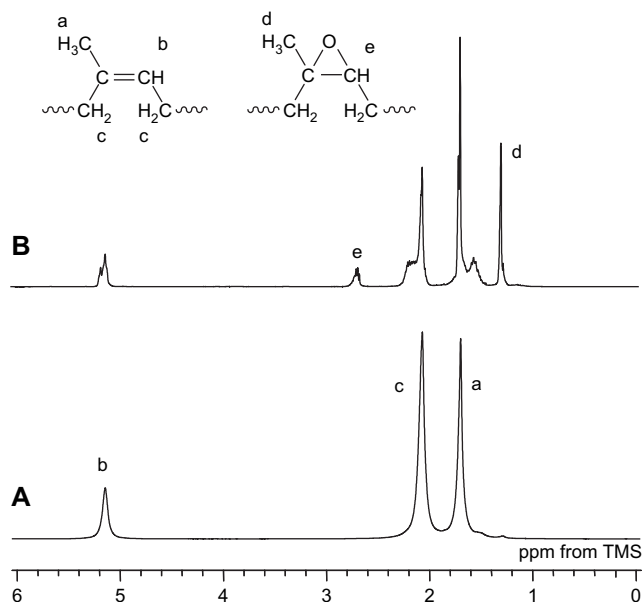


Fig. 1. Typical ^1H NMR spectra for (A) DPNR and (B) LEDPNR.

The chemical shifts for LEDPNR were estimated by using Furst's method [10,11] with respect to the triad sequences. The estimated chemical shifts are tabulated in Table 2. The unknown signals at 1.55 and 1.68 ppm could be attributed to methylene proton of the epoxidized unit and the methyl proton of the isoprene unit, respectively, while both signals at 2.05 and 2.15 ppm could be attributed to methylene proton of the isoprene unit.

To confirm the assignment, ^{13}C NMR measurement was applied to LEDPNR. Fig. 3 shows ^{13}C NMR spectra for DPNR and LEDPNR. In the spectrum for DPNR, five signals were appeared at 23.3, 26.2, 32.0, 125 and 135 ppm, which were assigned to C^5 , C^4 , C^1 , C^3 and C^2 , respectively. After

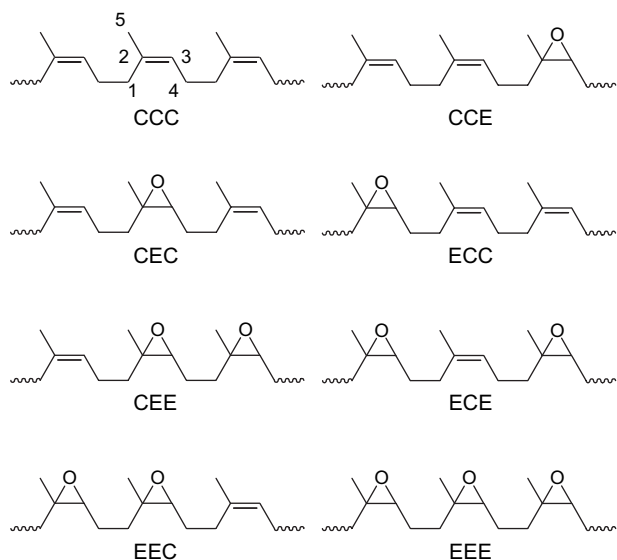


Fig. 2. All triad sequences of epoxidized units (E) and of isoprene units (C) in partially epoxidized natural rubber.

Table 2

Estimated chemical shift for LEDPNR by Furst's method

Chemical shift (ppm)	Estimated chemical shift (ppm)	Assignments by estimation
1.29	1.31	E^5
1.55	1.38	CE^1C , CE^1E , EE^1C , EE^1E , CE^4C , CE^4E , EE^4C , EE^4E
1.68	1.42	C^5
2.05	1.96	EC^1C , EC^1E , CC^4E , EC^4E
2.15	2.00	CC^1C , CC^1E , CC^4C , EC^4C
2.7	2.51	E^3
5.1	5.20	C^3

epoxidation, many signals appeared in aliphatic region and two signals appeared at 60.5 and 64 ppm. These new signals were assigned, as shown in Table 3, according to Bradbury and Perera [5]. The assigned signals in the aliphatic region of the ^{13}C NMR spectrum may be correlated to the signals in the ^1H NMR spectrum by observing spin coupling between ^{13}C and ^1H .

Fig. 4 shows HETCOR spectra for LEDPNR. The ^{13}C signals at 125 and 64 ppm, which were assigned to methine carbon of the isoprene unit and the epoxidized isoprene unit, were correlated to the ^1H signals at 5.1 and 2.7 ppm, respectively. This is consistent with the previous assignment, which was performed by 1D NMR spectroscopy. Fig. 5 shows expanded HETCOR spectra for LEDPNR. The ^1H signals at 1.29, 1.55, 1.68, 2.05 and 2.15 ppm were correlated to the ^{13}C signals, respectively, as shown in Table 3. In the previous investigations, as reported by Bhattacharjee et al. [1] and Thames and Gupta [2], the signals at 1.55 and 2.15 ppm had not been assigned, and the assignment of the signal at 1.68 ppm was inconsistent with each other. However, in the present study, the ^1H signals at 1.55, 1.68, 2.05 and 2.15 ppm in the ^1H NMR spectrum may be assigned: that is,

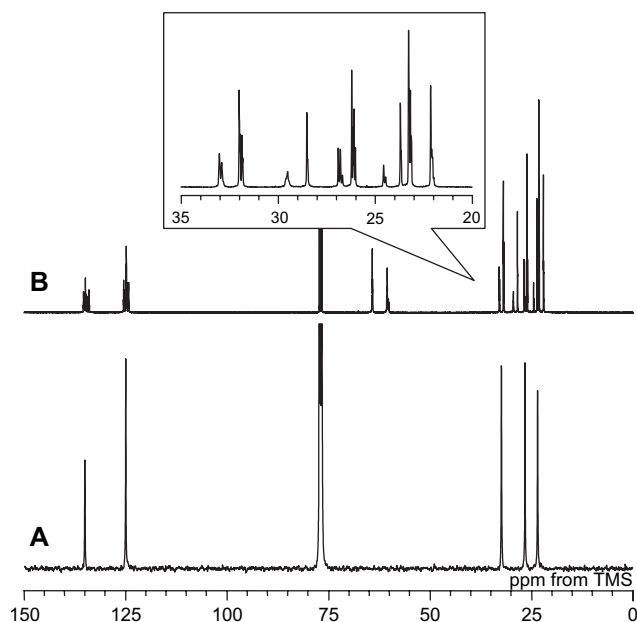


Fig. 3. ^{13}C NMR spectra for (A) DPNR and (B) LEDPNR.

Table 3
Assignments for ^{13}C NMR spectrum and spin coupling appeared in HETCOR spectra for LEDPNR

Chemical shift (ppm)	Assignments	δ for spin coupled ^1H signals in HETCOR spectra (ppm)
22.1	E^5	1.29
23.3	C^5	1.68
23.7	CC^4E , EC^4E	2.15
24.6	CE^4E , EE^4E	1.68
26.2	CC^4C , EC^4C	2.05
26.9	CE^4C , EE^4C	1.55
28.5	EC^1C , EC^1E	2.15
29.5	EE^1C , EE^1E	1.55
32.0	CC^1C , CC^1E	2.05
33.0	CE^1C , CE^1E	1.55
60.5	E^2	—
64	E^3	2.7
125	C^3	5.1
135	C^2	—

1.55 ppm assigned to CE^4C , EE^4C , EE^1C , EE^1E , CE^1C and CE^1E proton, 1.68 ppm to C^5 , CE^4E and EE^4E proton, 2.05 ppm to CC^4C , EC^4C , CC^1C and CC^1E proton, and 2.15 ppm to CC^4E , EC^4E , EC^1C and EC^1E proton.

HMBC measurement was made of LEDPNR to confirm the assignments shown in Table 4. HMBC spectra for LEDPNR are shown in Fig. 6. The ^1H signals at 1.55, 1.68, 2.05 and 2.15 ppm in the HMBC spectra were correlated to the ^{13}C signals, as shown in Table 4. The ^1H signal in the HMBC spectra is correlated with the ^{13}C signal that is one or two atom apart from the directly linked carbon atom. Therefore, the ^1H signals at 1.55, 1.68, 2.05 and 2.15 ppm in the ^1H NMR spectrum were assigned: that is, 1.55 ppm assigned to CE^4C , EE^4C , EE^1C , EE^1E , CE^1C and CE^1E proton, 1.68 ppm to C^5 , CE^4E and EE^4E proton, 2.05 ppm to CC^4C , EC^4C , CC^1C and CC^1E proton, and 2.15 ppm to CC^4E , EC^4E , EC^1C and EC^1E proton.

These assignments were supported with COSY spectra for LEDPNR. The COSY spectra are shown in Fig. 7. Since the cross peaks appeared between the signal at 1.55 ppm and at 2.15 ppm, it was confirmed that the signal at 1.55 ppm was

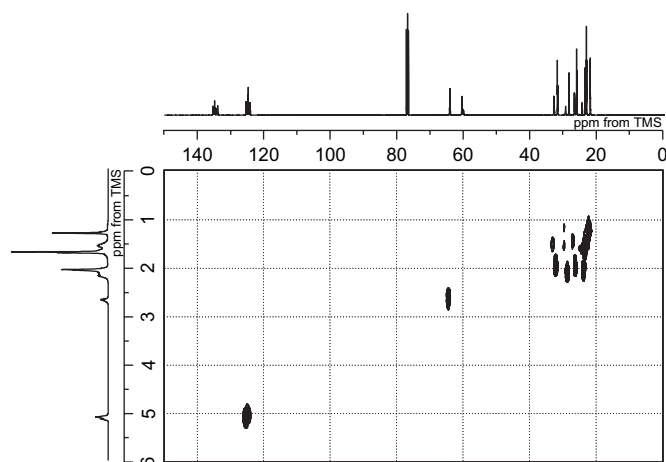


Fig. 4. HETCOR spectra for LEDPNR.

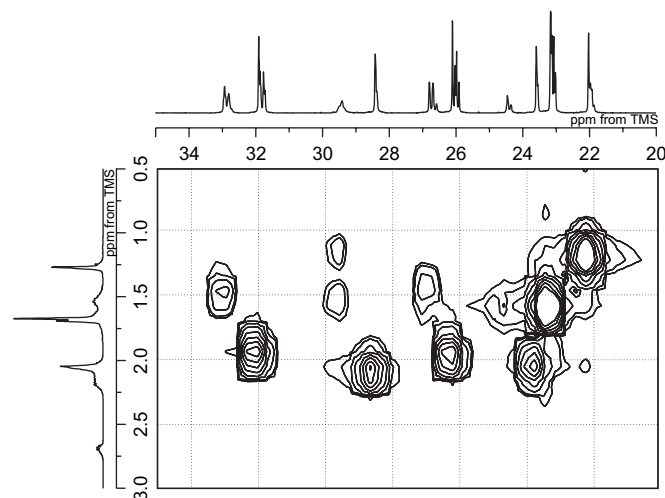


Fig. 5. Expanded HETCOR spectra for LEDPNR.

attributed to methylene proton of epoxidized unit neighbor with isoprene unit.

3.2. Characterization of epoxidized squalene as a model compound

The assignments for methylene proton are inconsistent with the assignments made by Bhattacharjee et al. [1] and Thames and Gupta [2]. To prove the assignments, epoxidized squalene as a model for epoxidized natural rubber was characterized through NMR spectroscopy. Fig. 8 shows ^1H NMR spectra for squalene and partially epoxidized squalene. In the spectrum for squalene, signals characteristic of *trans*-1,4-isoprene unit appeared at 1.65, 1.7, 2.1 and 5.1 ppm, which were assigned according to Aerts and co-workers [12]. The assignments are indicated in Fig. 8. After epoxidation of squalene, new signals appeared at 1.22, 1.26 and 2.7 ppm, which were assigned as indicated in Fig. 8. The epoxy group content of partially epoxidized squalene, Y_{epoxy} , was estimated from the intensity ratio of the signals at 2.7 and 5.1 ppm, as in the following equation:

$$Y_{\text{epoxy}} = \frac{I_{2.7}}{I_{2.7} + I_{5.1}} \times 100 \quad (2)$$

Table 4
Assignments for ^1H NMR spectrum and spin coupling appeared in HMBC spectra for LEDPNR

δ for ^1H (ppm)	Assignments	δ for spin coupled ^{13}C in HMBC spectra (ppm)
1.29	E^5	29.5, 33.0, 60.5, 64
1.55	CE^4C , EE^4C , EE^1C , EE^1E , CE^1C , CE^1E	22.1, 23.7, 24.6, 28.5, 60.5, 64, 125, 135
1.68	C^5 , CE^4E , EE^4E	28.5, 29.5, 32.0, 60.5, 64, 125, 135
2.05	CC^4C , EC^4C , CC^1C , CC^1E	23.3, 26.2, 32.0, 125, 135
2.15	CC^4E , EC^4E , EC^1C , EC^1E	23.3, 26.9, 33.0, 60.5, 64, 125, 135
2.7	E^3	22.1, 24.6, 26.9, 28.5, 60.5
5.1	C^3	23.3, 23.7, 26.2, 28.5, 32.0, 33.0

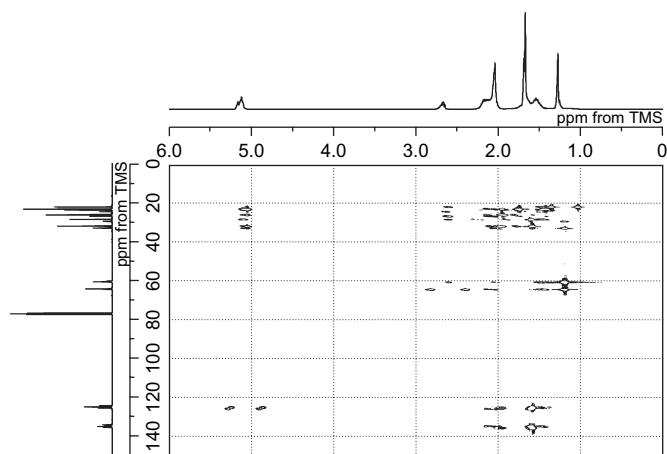


Fig. 6. HMBC spectra for LEDPNR.

where I is intensity of the signals and subscript numbers represent chemical shift (ppm). The estimated epoxy group content of partially epoxidized squalene was 20%. Therefore, at least one epoxy group was introduced into the each molecule as shown in Fig. 9.

Fig. 10 shows ^{13}C NMR spectra for squalene and partially epoxidized squalene. As for squalene, nine signals characteristic of the *trans*-isoprene unit appeared at 15.9, 17.6, 25.6, 26.6, 28.2, 39.6, 124, 131 and 135 ppm. After epoxidation, new signals appeared in the ^{13}C NMR spectrum. These signals are listed in Table 5. Among these signals, the signals at 18.6, 24.8, 27.3, 36.2, 58.0 and 63.9 ppm were assigned to a terminal epoxidized unit, as reported by Aerts and co-workers [12]. To assign the signals, DEPT measurements were carried out. DEPT spectra for partially epoxidized squalene are shown in Fig. 11. In DEPT 45 spectrum, the signals at 58.0 and 60.5 ppm disappeared, which were assigned to the quaternary

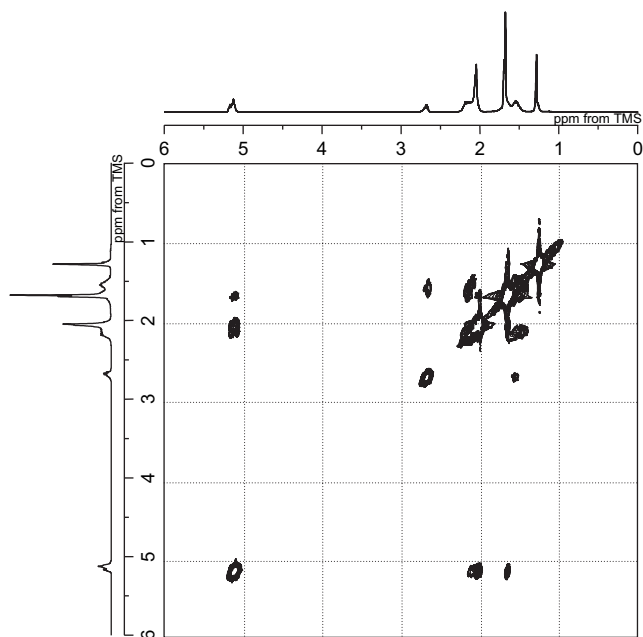
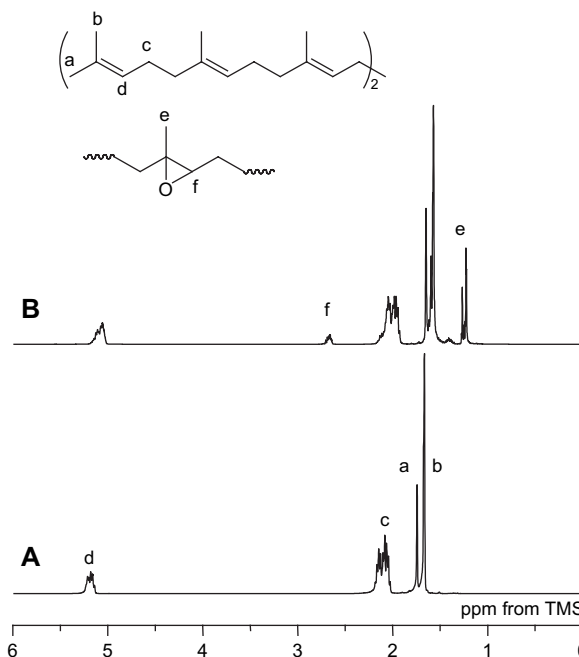


Fig. 7. COSY spectra for LEDPNR.

Fig. 8. ^1H NMR spectra for (A) squalene and (B) partially epoxidized squalene.

carbon of the terminal epoxidized unit and the internal epoxidized unit, respectively. Since the signals at 63.1 and 63.9 ppm appeared in DEPT 90 spectrum, these signals were assigned to the methine carbon of the internal epoxidized unit and the terminal epoxidized unit, respectively. In DEPT 135 spectrum, the upward signals at 16.5 and 18.6 ppm were assigned to the methyl carbon of the internal epoxidized unit and the *cis*-methyl carbon of the terminal epoxidized unit, respectively. Among the downward signals, the signals at 27.3 and 36.2 ppm will be assigned to the methylene proton of the terminal epoxidized unit and the methylene proton of the isoprene unit neighbor with the terminal epoxidized unit. The upward and downward signals at 24.8 ppm were found to be the *trans*-methyl carbon of the terminal epoxidized unit as well as the methylene carbon of the internal epoxidized unit or the isoprene unit neighbor with the internal epoxidized unit.

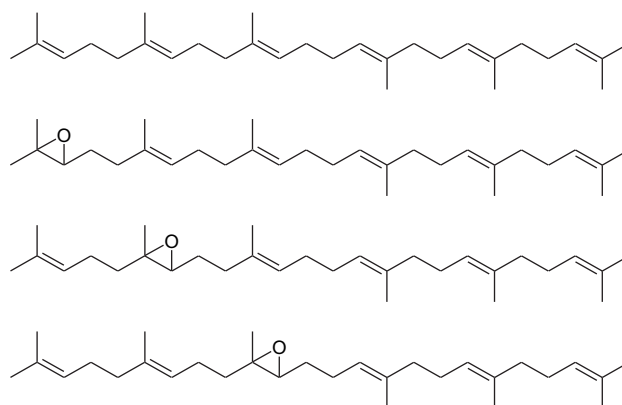


Fig. 9. Possible structure of epoxidized squalene.

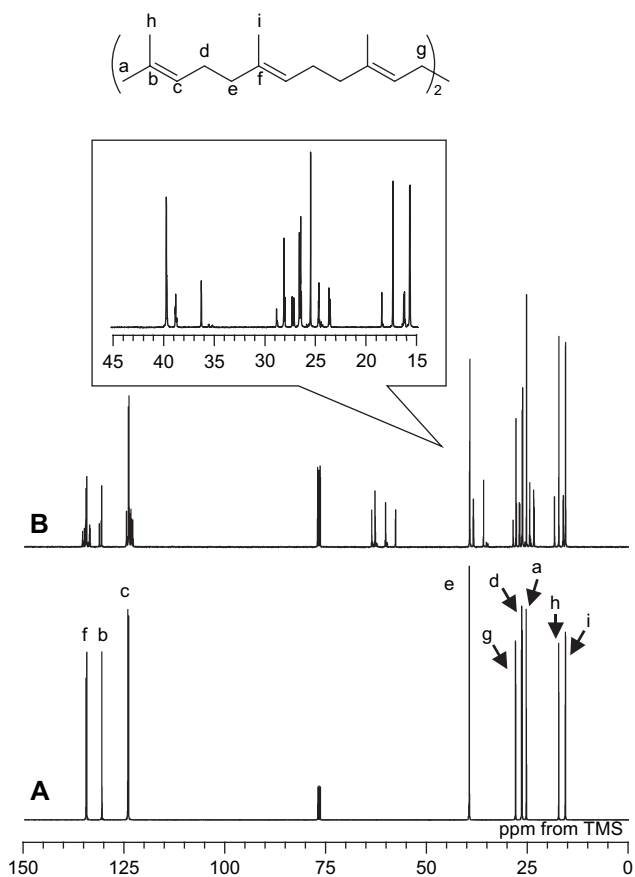


Fig. 10. ^{13}C NMR spectra for (A) squalene and (B) partially epoxidized squalene.

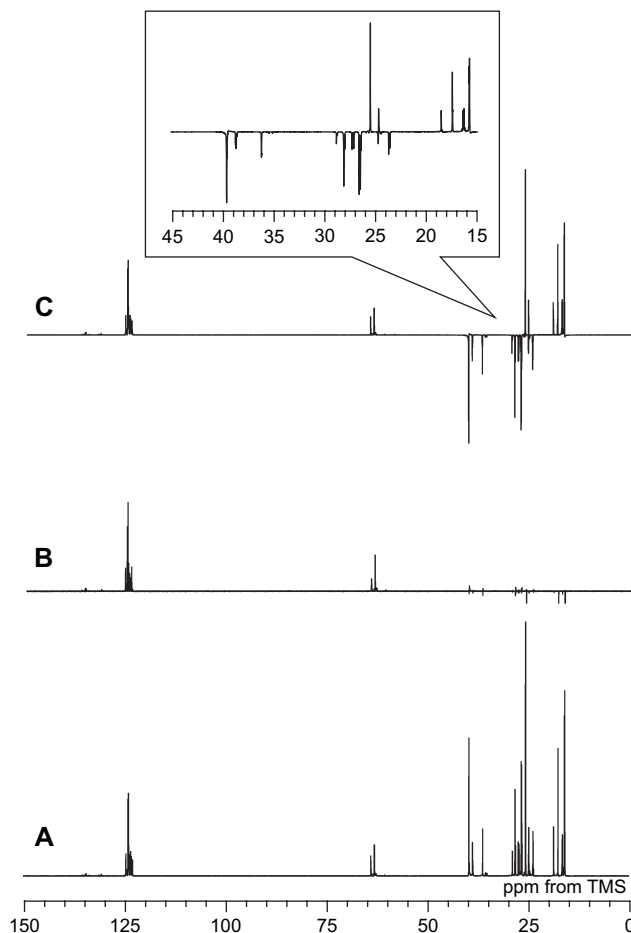


Fig. 11. (A) DEPT 45, (B) DEPT 90 and (C) DEPT 135 spectra for partially epoxidized squalene.

Table 5

Assignments for ^{13}C NMR spectrum, spin couplings appeared in HMBC spectra and results of DEPT measurement for partially epoxidized squalene

Chemical shift (ppm)	Results from DEPT ^a	δ for spin coupled ^1H signals in HMBC spectra (ppm)	Assignments
15.9	p	2.0, 5.1	$\text{CC}^5\text{C}-, \text{CCC}^5-$
16.5	p	1.64, 2.7	$\text{CE}^5\text{C}-, \text{CCE}^5-$
17.6	p	1.64, 5.1	$\text{C}^5\text{CC}-$
18.6	p	1.26, 2.7	$\text{E}^5\text{CC}-$
23.8	s	1.64, 5.1	$\text{C}^4\text{EC}-, \text{CC}^4\text{E}-$
24.8	p, s	1.22, 1.56, 1.64, 2.7, 5.1	$\text{E}^1\text{CC}-, \text{CEC}^1-$
25.6	p	1.56, 5.1	$\text{C}^1\text{CC}-$
26.6	s	2.0, 5.1	$\text{C}^4\text{CC}-, \text{CC}^4\text{C}-$
27.3	s	2.0, 2.7	$\text{E}^4\text{CC}-$
28.2	s	1.56, 2.0, 2.7, 5.1	$\text{CCC}^4-, \text{CCE}(\text{C}^4\text{CC})$
28.9	s	2.7	$\text{CE}^4\text{C}-, \text{CCE}^4-$
36.2	s	1.56, 1.64, 2.7, 5.1	$\text{EC}^1\text{C}-$
38.7	s	1.22, 2.0, 2.7	$\text{CE}^1\text{C}-, \text{CCE}^1-$
39.6	s	1.56, 1.64, 2.0, 5.1	$\text{CC}^1\text{C}-, \text{CCC}^1-$
58.0	q	1.22, 1.26, 1.56, 2.7	$\text{E}^2\text{CC}-$
60.5	q	1.22, 1.56, 1.64, 2.0, 2.7	$\text{CE}^2\text{C}-, \text{CCE}^2-$
63.1	t	1.22, 1.26, 1.56, 1.64, 2.0	$\text{CE}^3\text{C}-, \text{CCE}^3-$
63.9	t	1.22, 1.26, 1.64, 2.0	$\text{E}^3\text{CC}-$
124	t	1.56, 1.64, 2.0	$\text{C}^3\text{CC}-, \text{CC}^3\text{C}-, \text{CCC}^3-$
131	q	1.56, 1.64, 2.0	$\text{C}^2\text{CC}-$
135	q	1.56, 2.0	$\text{CC}^2\text{C}-, \text{CCC}^2-$

^a p: primary, s: secondary, t: tertiary, q: quaternary.

Fig. 12 shows HMBC spectra for partially epoxidized squalene. The spin couplings are summarized in Table 5. One example for the assignment of the methylene carbon was as follows: the ^{13}C signal at 36.2 ppm, which was attributed to the methylene carbon of the terminal epoxidized unit, was correlated to the ^1H signals at 2.7 and 5.1 ppm. In the terminally epoxidized squalene, the methylene carbon which can be

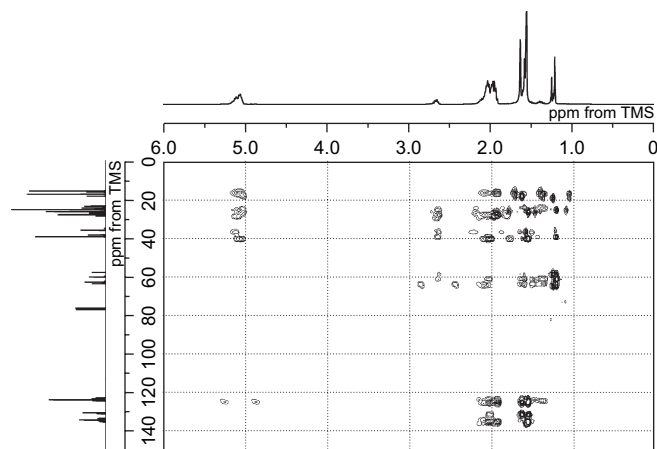


Fig. 12. HMBC spectra for partially epoxidized squalene.



Fig. 13. Expanded ^{13}C NMR spectrum for partially epoxidized squalene.

correlated to the ^1H signals at 2.7 and 5.1 ppm was one of the isoprene units neighbor with the terminal epoxidized unit. Therefore, the ^{13}C signal at 36.2 ppm was assigned to $\text{EC}^1\text{C}-$. The other signals were assigned in the similar way. These assignments are shown in Fig. 13.

Since the ^{13}C NMR spectrum for partially epoxidized squalene was assigned, HETCOR measurement was carried out. Fig. 14 shows HETCOR spectra for partially epoxidized squalene. The ^1H signal at around 1.6 ppm was correlated to the ^{13}C signals at 15.9, 17.6, 25.6, 27.3, 28.9 and 38.7 ppm, and the ^1H signal at 2.0 ppm was correlated to the ^{13}C signals at 23.8, 24.8, 26.6, 28.2, 36.2 and 39.6 ppm. The ^{13}C signals at

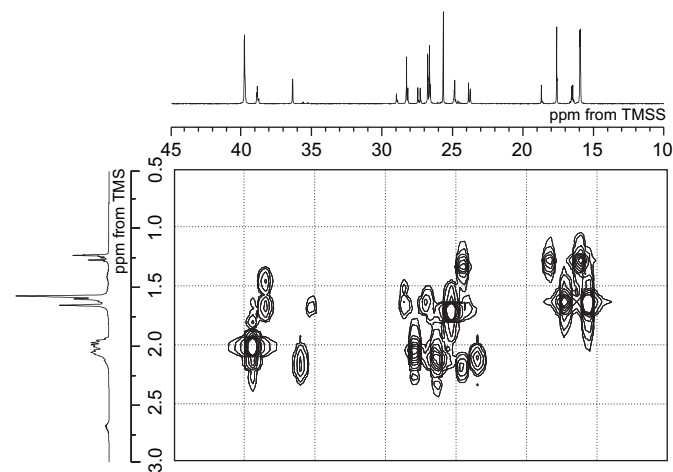


Fig. 14. HETCOR spectra for partially epoxidized squalene.

15.9, 17.6 and 25.6 ppm have been attributed to the methyl carbon of the isoprene unit and the signals at 27.3 and 28.9 ppm have been to the methylene carbon of the epoxidized isoprene unit. Consequently, the ^1H signal at around 1.6 ppm was assigned to the methyl proton of the isoprene unit and the methylene proton of the epoxidized isoprene unit. Further, the ^1H signal at 2.0 ppm, which was correlated to the methylene carbon of the isoprene unit, was assigned to the methylene proton of the isoprene unit.

3.3. Comparison of LEDPNR with partially epoxidized squalene

^1H NMR spectra for LEDPNR and partially epoxidized squalene are shown in Fig. 15. In the spectrum for partially epoxidized squalene, the signal at 1.6 ppm was assigned to the methyl proton of the *trans*-isoprene unit as well as the methylene proton of the epoxidized *trans*-isoprene unit, as mentioned above, and the signal at 2.0 ppm was assigned to the methylene proton of the *trans*-isoprene unit. On the other hand, for LEDPNR, the signal at 1.55 ppm was assigned to the methylene proton of the epoxidized *cis*-isoprene unit, the signal at 1.68 ppm was assigned to the methyl proton of the *cis*-isoprene unit as well as the methylene proton of the epoxidized *cis*-isoprene unit, and the signals at 2.05 and 2.15 ppm were assigned to the methylene proton of the *cis*-isoprene unit. The chemical shift of the signals in the spectrum for partially epoxidized squalene was quite similar to that of LEDPNR. Thus, the signal at 1.68 ppm in the spectrum for LEDPNR is proved to be the methyl proton of the *cis*-isoprene unit and the methylene proton of the epoxidized *cis*-isoprene unit. In

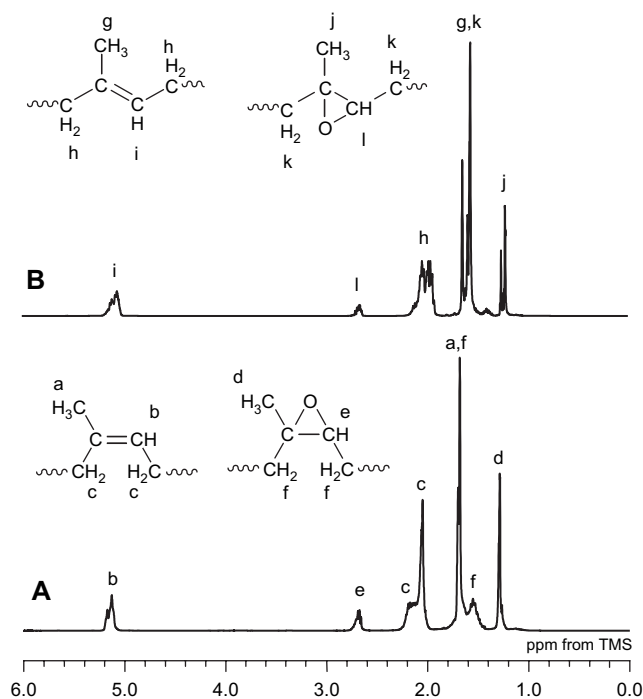


Fig. 15. ^1H NMR spectra for (A) LEDPNR and (B) partially epoxidized squalene.

addition, the signals at 2.05 and 2.15 ppm are proved to be the methylene proton of the *cis*-isoprene unit.

Since the signals in the aliphatic region were assigned as shown in Fig. 15, the epoxy group content, Z_{epoxy} , was estimated from the intensity ratio of the signals at 1.29 and 2.0–2.2 ppm, as in the following equation:

$$Z_{\text{epoxy}} = \frac{I_{1.29}}{I_{1.29} + I_{2.0-2.2} \times 3/4} \times 100 \quad (3)$$

where I is intensity of the signals and subscript numbers represent chemical shift (ppm). The estimated epoxy group content of LEDPNR used in the present study was 32%. This is similar to 33% estimated by Eq. (1). Therefore, it was proved that the signals in the aliphatic region of the ^1H NMR spectrum were correctly assigned through 2D NMR spectroscopy.

4. Conclusion

Epoxidized natural rubber was characterized by 1D- and 2D-NMR spectroscopy such as HETCOR, COSY and HMBC measurement. The unknown signals at 1.55, 1.68, 2.05 and 2.15 ppm in the ^1H NMR spectrum were assigned: that is, 1.55 ppm assigned to the methylene proton of the

epoxidized isoprene unit, 1.68 ppm to the methyl proton of the isoprene unit as well as the methylene proton of the epoxidized isoprene unit, and 2.05 and 2.15 ppm to the methylene proton of the isoprene unit. The assignment was proved with partially epoxidized squalene as a model compound.

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