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# *Why Rubber Trees Produce Polyisoprene – a Possible Role of Natural Rubber in the* Hevea *Tree*

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A possible role of rubber in the *Hevea brasiliensis* tree was presumed based on the analysis of structural changes of rubber during storage in the rubber tree as latex. The rubber obtained from a previously untapped mature-tree, a so-called virgin mature-tree (Vir-NR), contained a gel fraction higher than 80%. The gel fraction showed almost the same structure as a crosslinked rubber prepared from fresh latex in the presence of peroxide, with the molecular weight of a primary segment between crosslinks ($\overline{M}_c$) of $3 \times 10^3$. The sol fraction from Vir-NR was an oxidative degraded product containing aldehyde and epoxide groups. The gel fraction from a regularly tapped mature-tree (Reg-NR) was less than 3% and was loosely crosslinked with hydrogen bondings and ester linkages, having $\overline{M}_c$ of $7 \times 10^5$. These indicate that rubber accepts radicals, to form C-C crosslinking and partly oxidative degradation products in laticiferous tubes of *Hevea* tree during storage, suggesting a possible role of rubber as a scavenger of hydroxy radicals in latex

Some investigators regard natural rubber (NR) as a waste product of rubber trees or a useful protective product to avoid animal attack or disease incidence[1]. Another assumption that rubber acts as a reserve energy supplier seems most unlikely, because polyisoprene is presumed to be a product of secondary metabolism which has no apparent biological function[2]. However, it may become feasible to investigate the role of NR in *H brasiliensis* tree by chemical or enzymatic reaction analysis of polyisoprene *in vivo*.

Rubber obtained from the regularly tapped tree is almost completely soluble in good solvents[3], while the gel fraction increases with increasing preservation period of latex in the presence of ammonia[4]. Bloomfield[5] and Sekhar[6] have reported that virgin trees, which were never previously tapped, produced rubber containing high percentage of gel even from fresh latex. However, it was found that continuous tapping decreased the gel content and increased aldehyde groups[6]. These findings indicate that some reactions cause the gel formation during storage of latex *in vivo*.

There is, so far, no information on the structure of these gels. Thus, the present paper is an attempt to elucidate the structure of rubber obtained from virgin mature-trees and give comprehensive information on the possible role of polyisoprene in *H brasiliensis* trees.

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## MATERIALS AND METHODS

Fresh latex of *H. brasiliensis* was obtained by tapping several virgin mature-trees, growing at Prince of Songkla University's experiment station, Thailand. The latex was preserved by the addition of 1% sodium dodecyl sulphate, then adjusted to pH 9.2 by $Na_2HPO_4$, incubated with a proteolytic enzyme (KP 3939, Kao Co., Japan) under nitrogen atmosphere in the dark at 37°C for 24 h and centrifuged at 12 000 g for 30 min (×3). The deproteinised latex was coagulated by acetone and the rubber was purified by reprecipitation with methanol in toluene solution. Transesterification was carried out by treating 1% (w/v) toluene solution of rubber with 1 M freshly prepared sodium methoxide under nitrogen atmosphere in the dark at room temperature for 3 h. The rubber was further purified by acetone-extraction under nitrogen atmosphere in the dark for 36 h.

The rubber was dissolved in dried toluene to make 0.2% (w/v) and kept in the dark at room temperature without shaking or stirring for one week, then centrifuged at 10 000 g for 30 min to separate the gel. The resulting gel was dried *in vacuo* at room temperature and weighed to determine gel content. Molecular weight of sol fractions from all the samples was determined by GPC. The $\overline{M}_c$ was estimated by equilibrium swelling analysis according to the method of Mark[7]. The $^{13}$C-NMR measurements were done with a JEOL α-500 at 125 MHz at 50°C in deuterated chloroform with PD of 7 s. The $^{13}$C-NMR analyses of the gel fractions were carried out using a JEOL GX-400 at 100 MHz by Gated High-Power Decoupling (GHPD) and Magic Angle Spinning (MAS) measurement with PD of 7 s at 6 MHz spinning rate.

A model system of NR latex was prepared by incubating 30% DRC of fresh latex in the presence of 1% sodium dodecyl sulphate with *tert*-butyl hydroperoxide (2%, v/w) and tetraethylene pentamine (2%, v/w) under nitrogen atmosphere at 20°C in the dark for five days. The rubber obtained was coagulated with acetone and purified by acetone extraction for 36 h. The soluble and insoluble fractions were separated in a similar method as described above.

## RESULTS AND DISCUSSION

*Table 1* shows the contents of gel fraction and aldehyde group in Vir-NR and Reg-NR, before and after deproteinisation by a proteolytic enzyme and transesterification by freshly prepared sodium methoxide. Only Vir-NR contained aldehyde groups, which was reduced slightly by deproteinisation and significantly by transesterification. Since transesterification removes the fatty acid ester linked to rubber molecules at the terminating-end[8], most of the aldehyde groups are derived from oxidative degradation of unsaturated fatty acids and partly from rubber molecules. This result is inconsistent with the previous report[6]. The difference may be derived from the analytical method of the aldehyde group.

For mature trees, Vir-NR contains more than 80% gel fraction irrespective of the age of the tree in contrast to less than 3% for Reg-NR. Rubber from commercially obtained high ammonia latex (HA-latex), and solid pale crepe rubber (PC), contains more than 50% gel fraction. Part of gel from Reg-NR, HA-latex and PC is solubilised by the addition of a small amount of polar solvent to a good solvent and all of them are solubilised by transesterification with sodium methoxide[9]. This kind of gel is referred to as a soft-gel. We have presumed



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TABLE 1. GEL AND ALDEHYDE CONTENTS AS WELL AS MOLECULAR WEIGHT OF RUBBER FROM VIR-NR AND REG-NR

| Rubber sample | Gel content (%, w/w) | Aldehyde content (mmol/kg rubber) | $\overline{M}_w$* ($10^5$) | $\overline{M}_n$* ($10^4$) | $M_c$† ($10^3$) |
|---|---|---|---|---|---|
| Vir-NR | 80.2 | 12.5 | 6.0 | 8.8 | 2.5 |
| Vir-DP-NR[a] | 78.8 | 11.9 | 6.3 | 10.3 | 3.6 |
| Vir-DP-TE-NR[b] | 79.7 | 3.2 | 5.3 | 9.9 | 3.4 |
| Reg-NR | 2.2 | 0 | 25.0 | 27.0 | – |
| Reg-DP-NR[a] | 1.2 | 0 | 21.1 | 21.6 | – |
| Reg-DP-TE-NR[b] | 0 | 0 | 19.0 | 18.0 | – |

*The $\overline{M}_w$ and $\overline{M}_n$ of the sol fraction.

†The average $M_c$ of the gel fraction.

[a]Vir-DP-NR and Reg-DP-NR : Deproteinised rubber from virgin tree and regularly tapped mature tree, respectively.

[b]Vir-DP-TE-NR and Reg-DP-TE-NR : Transesterified rubbers from deproteinised latex of virgin tree and regularly tapped mature tree, respectively.

that NR consists of linearly, branched chains and pseudo-crosslinked molecules, in which the branching and crosslinking points are composed of proteins and phospholipids[10]. We have elucidated that deproteinisation and transesterification can decompose the above branch-points, respectively[9]. However, it is noteworthy that the gel fraction of Vir-NR was not solubilised even after these treatments. This indicates that almost all of Vir-NR is composed of a crosslinked polymer, a so-called hard-gel. The gel fraction showed the $\overline{M}_c$ values as low as about $3 \times 10^3$, corresponding to a highly crosslinked rubber. In contrast, the soft-gel from HA-latex and PC showed the $\overline{M}_c$ values[8] of about $7 \times 10^5$.

A model rubber prepared *in vitro* for reproducing the crosslinking reaction in the rubber tree contained 90% gel fractions with $\overline{M}_c$ of $2.5 \times 10^3$. The gel content was insignificantly changed before and after deproteinisation and transesterification. *Figure 1* shows the solid-state $^{13}$C-NMR spectra of the gel fractions from Vir-NR and crosslinked latex. In both spectra, each of the small signals showed almost the same chemical shift. The assignment of these signals was done by estimating chemical shifts of the carbon atoms around the presumed crosslinking points according to the method of Bremser *et al.*[11] For example, signals at δ 52.16, 45.84 and 15.92 were assigned to the quaternary methine (—C(|)—CH<) and methyl carbon atoms characteristic of crosslinking-points. Four small signals at δ 42.94, 46.91, 147.36 and 109.38 were due to CH, CH, C and $CH_2$, which derived from the branch-point adjacent to a

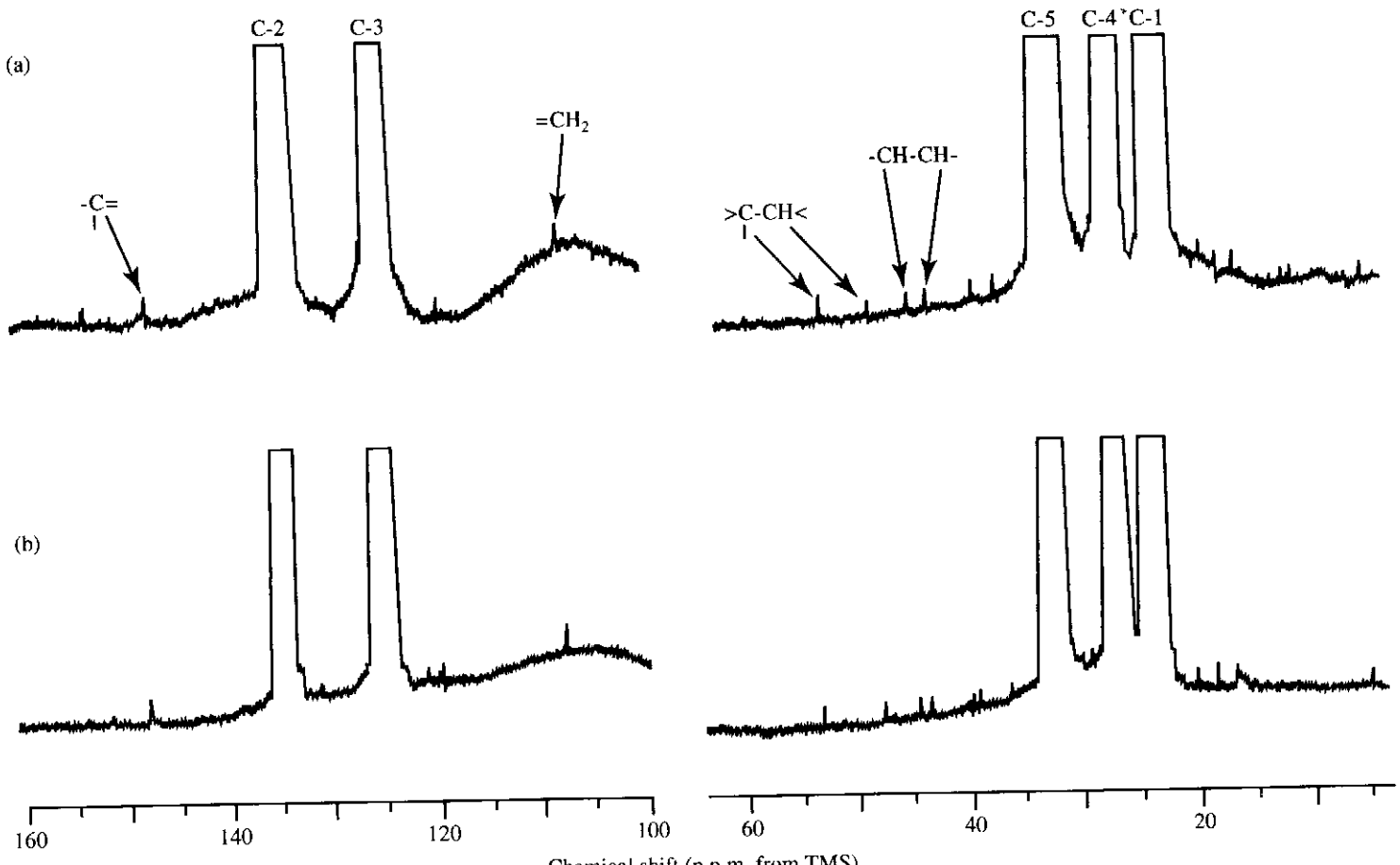


*Figure 1. Solid state $^{13}C$-NMR spectrum of transesterified gel fraction of rubbers from (a) Vir-NR and (b) crosslinked latex.*

vinylidene group (>CH—CH—C=$CH_2$). These assignments are in accordance with those interpreted by Patterson and Koenig[12] for polyisoprene crosslinked by γ-irradiation. These findings clearly indicate that the gel fraction in Vir-NR is a crosslinked polyisoprene similar to the radically cured rubber.

The sol fraction, less than 20% in Vir-NR, was low molecular-weight rubber with $\overline{M}_n$ of about $9 \times 10^4$, which was about one-fourth of that of Reg-NR. It showed the $^{13}$C-NMR spectrum similar to oxidative degraded products from NR having aldehyde and epoxide groups[13] (*Figure 2*). The sol fraction from the crosslinked latex showed almost the same spectrum as that of Vir-NR. It is noteworthy that the signals at δ 39.48, 39.35, 16.54, 119.0 and 68.37 are assigned to $CH_2$, C, CH, $CH_2$ and $CH_3$ in the hydroperoxide structure (—$CH_2$—C($CH_3$)=CH—$CH_2$—O—O—R), respectively. These findings show that the sol fraction in Vir-NR is a by-product formed in the course of the reaction of fresh latex with peroxide.

It is generally accepted that polyisoprene is susceptible to oxidation by air under a mild condition to form hydroperoxides. The decomposition of these hydroperoxides forms oxy- and peroxy-radicals, which abstract allylic hydrogens to form free-radicals on the polymer chain. These reactions result in the auto-oxidation process[14] and preferentially crosslinking reaction, if the oxygen concentration is low. Latex contains various kinds of antioxidants such as tocotrienols, allo-ocimene, Vitamin A or β-carotene[15]. These are much more susceptible to oxidation rather than the polyisoprene molecules. However, after their consumption during prolonged storage in the tree, the *in situ* rubber should facilitate the crosslinking reaction.

The phenomenon observed in this study was common to the rubbers from Vir-NR independent of age once the tree has reached maturity. The combination of the basic knowledge and the findings in this study would give a new idea on a possible role of *cis*-1,4 - polyisoprene in the rubber tree, *i.e.*, the polyisoprene molecules will act as a radical scavenger *in situ*. In addition, we have also analysed the so-called wild rubbers occurring as latex from higher plants such as Jelutong (*Dyera costulata*), Sorvinha (*Coma utilis*) or Indian Laurel (*Ficus elastica*)[16]. Rubber from these trees also contain very high percentage of hard-gel. The sol fraction of these rubbers showed only five major signals due to *cis*-1,4 isoprene units and small signals due to the oxidative degradation of double bonds such as epoxide and carbonyl groups, as can be observed in the spectra of the sol fraction of rubber from *Hevea* tree. The details on the structure of wild rubber will be discussed in a subsequent paper. This will be supporting evidence for the above supposition, by considering the fact that these wild rubbers are obtained from trees which were never tapped previously.

## CONCLUSION

The gel fraction predominant in the rubber from Vir-NR was confirmed to be a highly crosslinked rubber with the $\overline{M}_c$ of $3.0 \times 10^3$, similar to a model rubber cured with peroxide in latex by radical reaction. The sol fraction of rubber from Vir-NR was a low molecular-weight rubber formed by oxidative degradation. A possible role of *cis*-1,4-polyisoprene is presumed to be a radical acceptor in the rubber tree.

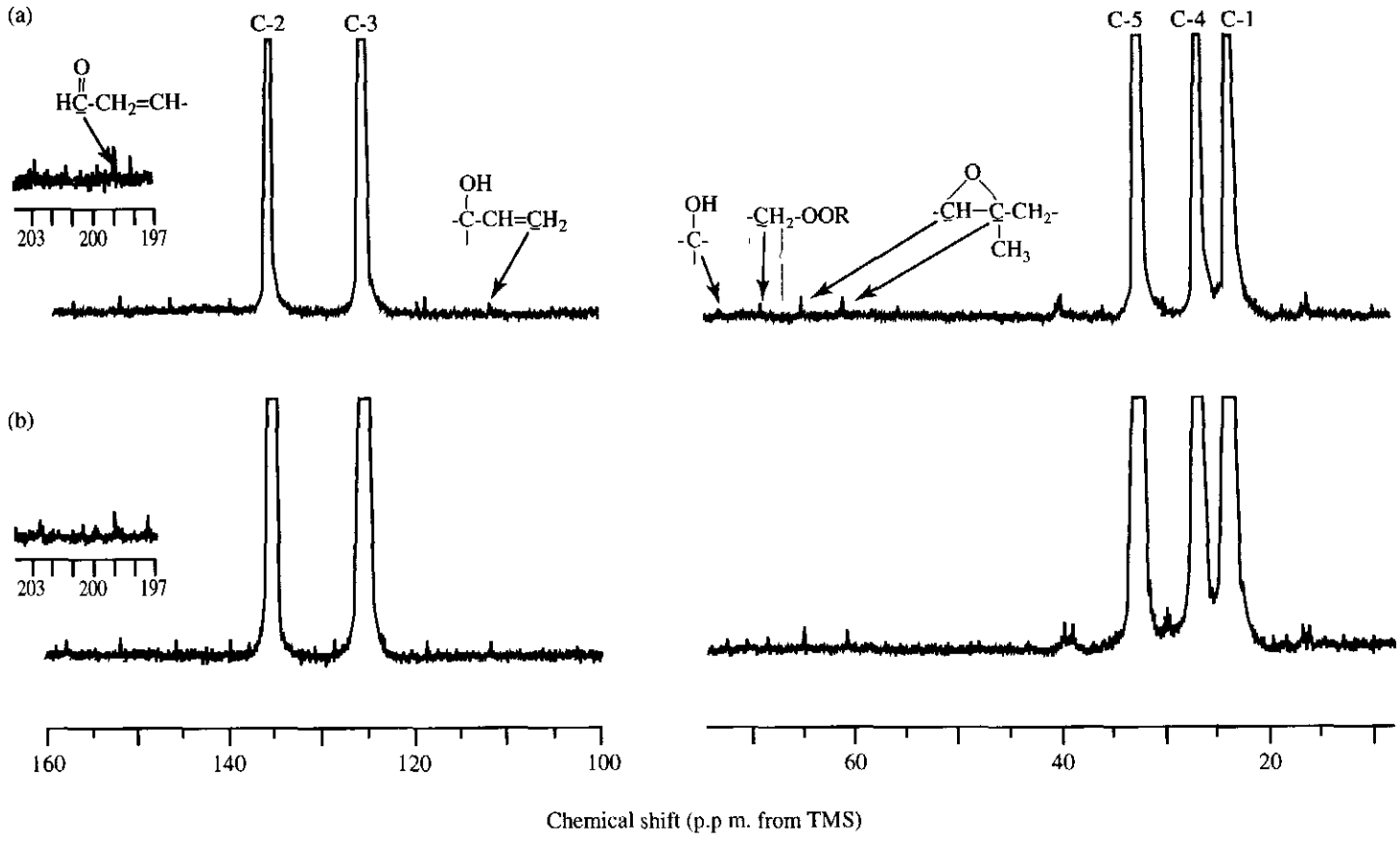


*Figure 2. $^{13}$C-NMR spectrum of sol fraction of rubbers from (a) Vir NR and (b) crosslinked latex.*

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