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Structural Characterisation of Naturally Occurring Trans-*Polyisoprenes*

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The structure of trans-*polyisoprene isolated from Chicle latex and Gutta percha latex was analysed by* ^{13}*C-NMR spectroscopy. The* trans-*polyisoprene from Chicle latex showed the* ^{13}*C-NMR signals characteristic of the dimethylallyl terminal unit (ω-terminal) and* trans-*isoprene unit terminated with hydroxyl and ester groups (α-terminal). The ester terminal group was not detected in the* trans-*polyisoprene from Chicle resin. The degree of polymerisation estimated from the intensity ratio of the signals from internal* trans *units and ω-terminal group was in fair agreement with the value obtained by GPC-LALLS measurement. However, the intensity ratio of signals from the ω-terminal and α-terminal groups was found to be 1/0.76, indicating the loss of a part of α-terminal in the latex sample. The* trans-*polyisoprene from Gutta percha latex showed a similar* ^{13}*C-NMR spectrum as that from Chicle. The loss of the α-terminal was also observed for the* trans-*polyisoprene from Gutta percha latex. It was presumed that a chemical or biochemical modification of α-terminal groups occurred during storage of the latex.*

Few species of higher plants have been shown to produce polyisoprene of *trans*-1, 4 configuration. Gutta percha from *Palaquium gutta* and Balata from *Mamusops balata* are typical *trans*-polyisoprene occurring as latex. *Achras sapota* is known to contain two types of polyisoprenes in *trans* and *cis* configurations, called Chicle. It was proved by ^{13}C-NMR analysis that Chicle polyisoprene is a mixture of *trans*-polyisoprene and *cis*-polyisoprene[1,2].

The authors have investigated the terminal groups and alignment of isoprene units in naturally occurring *cis*-polyisoprenes by using the ^{13}C-NMR method which was established on the basis of ^{13}C-NMR analysis of acyclic terpenes and polyprenols[3]. It has been found that *cis*-polyisoprenes isolated from the leaves of Goldenrod and Sunflower are a high molecular weight homologue of polyprenol consisting of a dimethylallyl terminal unit, of about three *trans* units, and a long sequence of *cis* units terminated with hydroxyl or ester group aligned in that order[4,5,6]. However, *cis*-polyisoprenes occurring as latex such as *Hevea* rubber, Jelutong rubber, and Sorvinha rubber were found to have both terminal groups different from polyprenols and terpenes[7]. On the other hand, *cis*-polyisoprene from mushroom was found to be a high molecular weight homologue of two-*trans* polyprenol containing a dimethylallyl unit and an ester terminal group, despite the fact that it exudes from sporophores as latex[8]. In addition, a significant decrease of both terminal units was observed when sporophores were stored a few days after harvesting. This implies that the absence of both terminal groups in rubbers occurring as latex correlates with the period of storage in laticiferous cells and that after collection.

This paper reports on the structural characterisation of *trans*-polyisoprenes obtained from the latices of Chicle and Gutta percha by using the ^{13}C-NMR method. The quantities

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of both terminal units were checked by comparing the degree of polymerisation from the ^{13}C-NMR measurement with that from the GPC-LALLS.

EXPERIMENTAL

Fresh Chicle latex collected from a single tree in Quintana-Roo, Mexico was subjected to purification two weeks after collection by tapping. The resinous fraction was removed by Soxhlet extraction with acetone from the partially coagulated latex sample. Commercially obtained Chicle resin was utilised as a reference sample. Pure *trans*-polyisoprene was separated from *cis*-polyisoprene by repeated recrystallisation from hexane solution at 40°C. Gutta percha latex was collected from a single tree in Sumatra, Indonesia. Three weeks after collection, spontaneously coagulated Gutta percha latex, was purified by reprecipitation from toluene solution with methanol, followed by Soxhlet extraction with acetone under nitrogen atmosphere. *Trans*-polyisoprene from Gutta percha latex was further purified by repeated recrystallisation in hexane. Low molecular weight fractions of Gutta percha latex were obtained by fractional precipitation in toluene/methanol. GPC-LALLS measurements were done with a JASCO 880-PU high pressure pump and a TOSOH LS-8000 low-angle laser light scattering photometer (LALLS) equipped with a TOSOH RI-8011 detector. Molecular weights of *trans*-polyisoprene samples were determined by using standard polystyrene samples. The ^{13}C-NMR measurements were done on a JEOL FX-200 at 50.1 MHz in $CDCl_3$ at 50°C using a pulse repetition time of 6 s for 50° pulse.

RESULTS

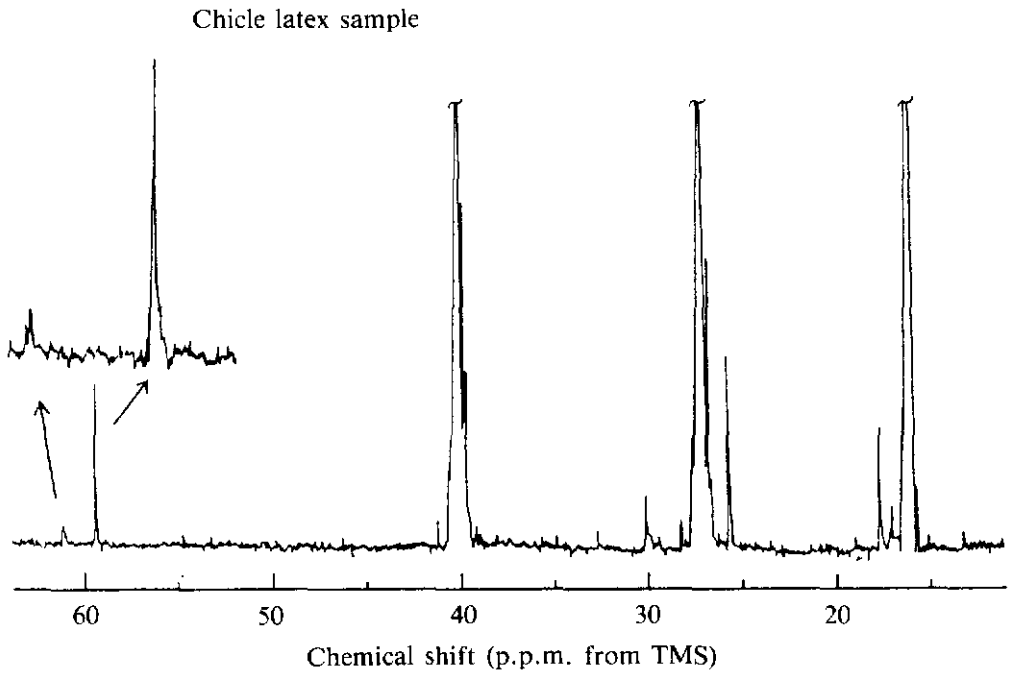
Trans-polyisoprene from Chicle Latex

The molecular weight of *trans*-polyisoprene from Chicle latex was found to be $\bar{M}w = 14 \times 10^3$ and $\bar{M}n = 7.8 \times 10^3$ by GPC-LALLS measurement. In the ^{13}C-NMR spectrum of Chicle *trans*-polyisoprene, small signals characteristic of the dimethylallyl terminal unit (ω-terminal) at 17.64 p.p.m. (C-5 CH_3) and 25.61 p.p.m. (C-1 CH_3) and the terminal *trans* unit linked to hydroxyl group (α-terminal) at 59.44 p.p.m. (C-4 CH_2OH) were observed as shown in *Figure 1*. Here, the carbon atoms in isoprene units including both the terminal units are designated as follows:

$$\begin{array}{c} C(5) \\ | \\ -C(1)-C(2)=C(3)-C(4)- \end{array}$$

In this spectrum, very small signals were detected at 61.32 and 29.70 p.p.m. The latter signal is assigned to the methylene carbon atoms in the $(CH_2)_n$ sequence. The former is assignable to a ω C-4 carbon linked to a fatty acid ester group. But other signals characteristic of saturated fatty acid ester groups, *i.e.* $OCOCH_2$ around 34 p.p.m. and methyl carbon signals around 14 p.p.m., were not detected due to trace amounts of the ester group. The intensity ratio of the signals at 61.32 and 59.44 p.p.m. indicates that about 15% of the α-terminal unit is esterified in the sample. These signals were also detected in the ^{13}C-NMR spectrum of *trans*-polyisoprene obtained from Chicle latex from different sources[9]. On the other hand, these signals were not observed in the spectrum of the sample from Chicle resin as shown in *Figure 1*. These findings indicate that the latex samples suffered a small amount of esterification during storage after collection or during storage in laticiferous cells.

The relative intensity of the *trans* C-1 methylene carbon signal and ω C-1 methylene carbon signal was found to be 140, which is in fair agreement with the degree of polymerisation of 120 determined by the GPC-LALLS measurement. The intensity ratio between the ω C-1 signal at 25.61 p.p.m. and the α C-4 signals at 61.32 and 59.44 p.p.m. was found to be 1/0.76. In the case of *trans*-polyisoprene from Chicle resin, which was usually coagulated directly after collection, the intensity ratios of the corresponding ω and α signals were found to be 1/1.05 for unfractionated samples and 1/0.91 for fractionated samples[2]. This indicates that *trans*-polyisoprene from Chicle latex has fundamentally the same structure as that from Chicle resin as shown below, although it is anticipated that a part of the hydroxyl terminal



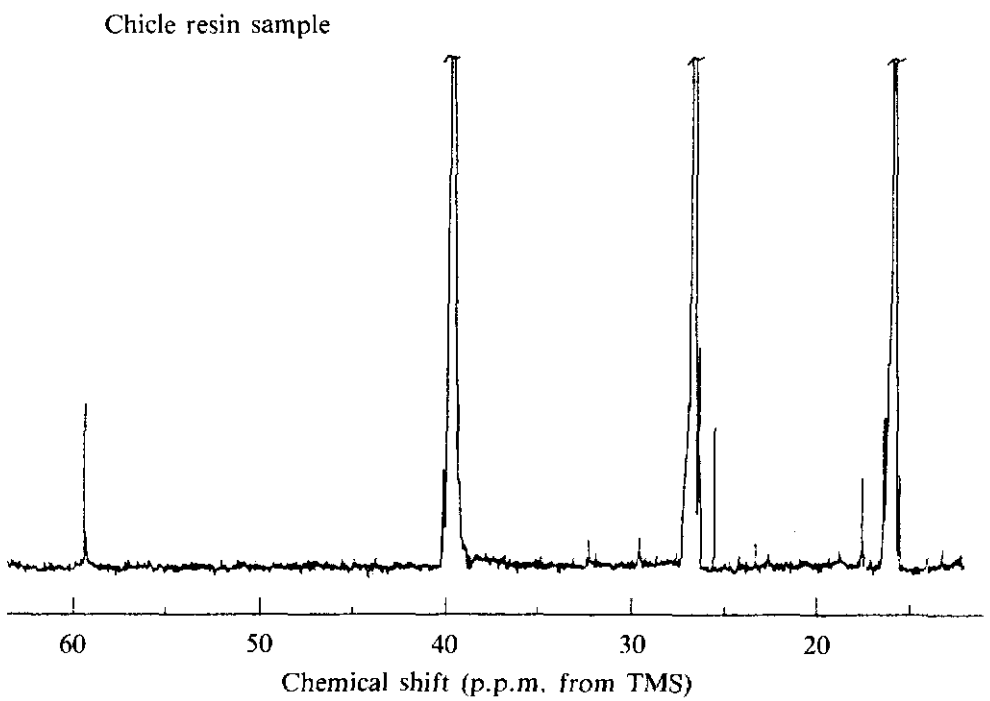
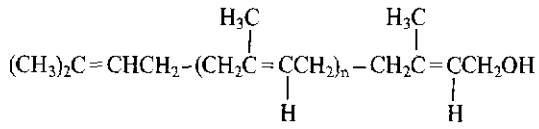


Figure 1. ^{13}*C-NMR spectrum of* trans-*polyisoprene from Chicle latex sample and Chicle resin sample.*

group would be modified during storage resulting in the loss of hydroxyl and/or ester groups:



Trans-polyisoprene from Gutta Percha Latex

The molecular weight of *trans*-polyisoprene from Gutta percha latex was found to be $\overline{M}w = 12 \times 10^4$ and $\overline{M}n = 48 \times 10^3$ by GPC-LALLS measurement. The ultimate fraction of fractional precipitation was subjected to ^{13}C-NMR analysis. The low molecular weight fraction showed fundamentally the same ^{13}C-NMR signals as Chicle *trans*-polyisoprene. As shown in *Figure 2*, the ω-terminal unit showed characteristic signals at 17.61 p.p.m. (C-5), 25.61 p.p.m. (C-1), and 131.05 p.p.m. (C-2). Similarly, the signals at 59.36 and 139.52 p.p.m. were assigned to the C-4 and C-2 carbon atoms in the α-terminal unit, respectively. Signals from an ester group were not detected in the spectrum.

The degree of polymerisation estimated from the intensity ratio between the *trans* C-1 CH_2 and α C-4 CH_2 signals was found to be 760, which is higher than that of 510 determined by GPC-LALLS. Although the accurate relative intensity of the α C-1 signal was not obtained due to a poor resolution of the spectrum, the height of the signal is apparently lower than that of the α C-4 signal. The loss of the α-terminal signal was also observed for a commercially obtained Gutta percha solid sample[9]. This may imply the presence of a similar modification of both terminal units as in the case of *cis*-polyisoprene occurring as latex[7]. Commercially obtained Gutta percha resin showed small signals arising from epoxide groups and *cis*-polyisoprene[9], while

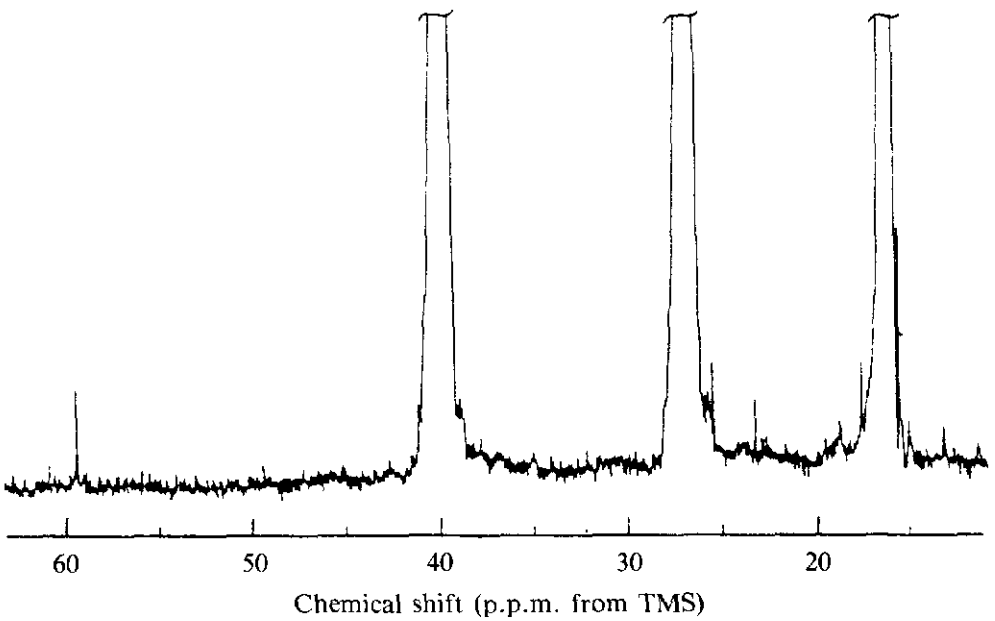


Figure 2. ^{13}C-NMR spectrum of low molecular weight trans*-polyisoprene from Gutta percha latex.*

no signal due to these impurities was detected in the latex sample. These findings demonstrate that the fundamental structure of *trans*-polyisoprene from Gutta percha is the same as that of Chicle *trans*-polyisoprene and the biosynthesis proceeds in a similar way.

DISCUSSION

The biosynthesis of *trans*-polyisoprene in Chicle is assumed to start from dimethylallyl pyrophosphate and proceeds by the successive addition of isopentenyl pyrophosphate to polyisoprenyl pyrophosphate[2]. The termination step is presumed to occur by dephosphorylation to form the hydroxyl α-terminal group. The presence of hydroxyl and ester α-terminal groups was clearly observed in *cis*-polyisoprene from the leaves of Sunflower formed in non-laticiferous cells[4,6]. Hydroxyl and ester terminal groups are in common to polyprenols, which consist of nine to twenty-three isoprene units widely distributed in higher plants[10]. On the other hand, these types of α-terminal groups and dimethylallyl terminal unit were not detected in *cis*-polyisoprene occurring as latex such as *Hevea* rubber[5,7,9]. The decrease of the dimethylallyl terminal unit as well as the α-terminal groups was also observed in *cis*-polyisoprene occurring as latex from mushroom during storage of sporophores[8]. These findings suggest that the α-terminal unit with hydroxyl or ester group is labile and readily chemically or biochemically modified in latex during storage resulting in loss of the functional groups.

A set of dimethylallyl and α-terminal units was detected in the case of *trans*-polyisoprene from Chicle. However, a part of the hydroxyl terminal group was replaced by the fatty acid ester group in Chicle latex stored two weeks after collection. Apparent reduction of α-terminal group was observed in the case of Gutta percha latex. These findings indicate that some chemical or biochemical reaction proceeds in latex to modify the terminal unit as in the case of *cis*-polyisoprene occurring as latex.

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