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STRUCTURE AND BIOSYNTHESIS OF *TRANS*-POLYISOPRENE FROM *EUCOMMIA ULMOIDES*

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Key Word Index—*Eucommia ulmoides*; Gutta Percha; Chicle; Balata; *trans*-polyisoprene; dimethylallyl diphosphate; geranylgeranyl diphosphate; solanesyl diphosphate.

Abstract—The structure of *trans*-polyisoprene from both bark and leaves of *Eucommia ulmoides* was analysed by ^{1}H NMR and ^{13}C NMR. Both spectra showed characteristic signals of the dimethylallyl-group, as well as terminal ester and diphosphate groups. The degree of polymerization estimated from the intensity ratio of signals from the internal *trans*-units and the dimethylallyl-group was in fair agreement with that determined by osmometry. The *trans*-polyisoprene from *E. ulmoides* is a high molecular-weight homologue of solanesol, consisting of the dimethylallyl-group, 60–3000 internal *trans*-units, and a terminal aliphatic fatty acid ester-group or a diphosphate group, aligned in that order. © 1997 Elsevier Science Ltd. All rights reserved

INTRODUCTION

Few species of higher plants have been shown to produce polyisoprene in the all *trans*-1,4 configuration [1]. Gutta Percha from *Palaqium gutta* and Balata from *Mimusops balata* are typical high molecular-weight *trans*-polyisoprenes occurring as latex [2]. *Achras sapota* produces low molecular-weight *trans*-polyisoprene and high molecular-weight *cis*-polyisoprene as a mixture in latex form [2, 3]. We have analyzed the structure of both the terminal groups and the alignment of isoprene units in *trans*-polyisoprene from Chicle [4], Gutta Percha and Balata [5] by ^{13}C NMR spectroscopy. These polymers are high molecular-weight homologues of acyclic terpenes such as geraniol, farnesol and geranylgeraniol, and a typical *trans*-oligoprenol such as solanesol. The mechanism of biosynthesis of these *trans*-polyisoprenes is presumed to proceed by successive addition of isopentenyl diphosphate (IDP) to dimethylallyl diphosphate (DMADP), geranyl diphosphate (GDP), farnesyl diphosphate (FDP) or geranylgeranyl diphosphate (GGDP), in the *trans*-configuration. The termination reaction is thought to be hydrolysis of the terminal diphosphate group to form a hydroxyl group, although no direct evidence for the presence of this diphosphate group has so far been found [4].

Eucommia ulmoides Oliv. is a higher plant known to produce *trans*-polyisoprene and is called Chinese Gutta Percha. It contains *trans*-polyisoprene in both leaves and bark. The dried bark is a traditional Chinese crude drug used as a tonic, analgesic and anti-hypertensive agent [6, 7]. This paper reports on the structural characterization of *trans*-polyisoprene from *E. ulmoides* Oliv., in connection with the mechanism controlling molecular weight.

RESULTS AND DISCUSSION

Polyisoprenes isolated from bark and leaves of *E. ulmoides* Oliv. were found to be a pure *trans*-polyisoprene by comparison of their ^{13}C NMR spectra with that of *trans*-polyisoprene obtained from Chicle (Fig. 1). The differential scanning calorimetry (DSC) results showed that the *trans*-polyisoprene extracted from leaves had a glass transition temperature, T_g, of $-62.8°$, and a melting temperature, T_m, of $58.3°$, which were close to those of *trans*-polyisoprene obtained from Gutta Percha (T_g, $-62.7°$, T_m, $60.9°$).

The molecular-weight distribution of the *trans*-polyisoprene extracted from bark showed a typical unimodal distribution, whereas the polymer obtained from leaves showed a bimodal distribution (Fig. 2). The number-average and weight-average molecular weight of the former were estimated to be $\bar{M}_n = 1.1 \times 10^5$ and $\bar{M}_w = 3.3 \times 10^5$, respectively, by calibration with standard polystyrenes. Similarly, those of the *trans*-polyisoprene extracted from leaves

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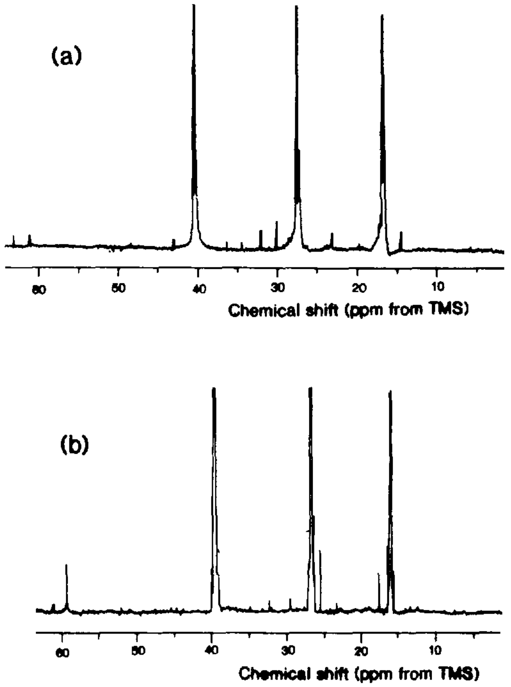
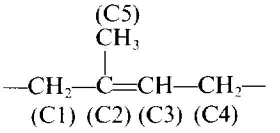


Fig. 1. ^{13}C-NMR spectra of *trans*-polyisoprene extracted from (a) *E. ulmoides* Oliv. and (b) Chicle.

were estimated to be $\bar{M}_n = 1.8 \times 10^4$ and $\bar{M}_w = 2.2 \times 10^5$. It is noteworthy that the high molecular-weight fraction, of leaf *trans*-polyisoprene, obtained by solvent fractionation, showed a similar molecular-weight distribution to that of the whole polymer extracted from bark, while the low molecular-weight fraction was characteristic of that obtained from leaves. The *trans*-polyisoprene from leaves was separated into four fractions by solvent fractionation, and that of lowest molecular-weight had $\bar{M}_n = 5.0 \times 10^3$, by vapour pressure osmometry (VPO).

The ^{1}H NMR spectrum of the lowest molecular-weight fraction of the polymer from leaves showed small signals characteristic of the dimethylallyl-group and terminal-groups (Fig. 3). Here, the protons and carbon atoms in the isoprene units, including the dimethylallyl-group, are designated as follows.



$$\underset{\text{(C1)}}{-\mathrm{CH_2}}-\underset{\text{(C2)}}{\overset{\overset{\text{(C5)}}{\mathrm{CH_3}}}{\overset{|}{\mathrm{C}}}}{=}\underset{\text{(C3)}}{\mathrm{CH}}-\underset{\text{(C4)}}{\mathrm{CH_2}}-$$

A small signal at δ 1.58 was formerly assigned to the methyl protons of *Z*-configuration in the dimethylallyl-group, which is termed the ω-terminal [8]. Two small signals corresponding to the methylene protons in the α-terminal-isoprene unit, were observed at δ 4.67 as a doublet signal, and at δ 4.36 as a triplet signal. The former was assigned to the terminal methylene protons, α-C-4 CH_2, esterified with a long-chain fatty acid. The other signals due to the long-chain fatty acid ester group were observed at δ 0.90 ($-CH_3$), 1.26 ($-CH_2-$) and 2.38 ($-CH_2CO_2-$). These were confirmed by comparison with the spectrum of solanesyl stearate as a model compound, which showed the corresponding signal at δ 4.67 as a doublet with $J = 6.74$ Hz, as shown in Fig. 4(a). The triplet signal centered at δ 4.36, was composed of two doublets with $J = 6.74$ Hz and $J = 5.94$ Hz by decoupling from the α-C-2 olefinic proton ($=CH$) at δ 5.49. This splitting suggested the presence of coupling from a remote nucleus of spin 1/2, such as ^{31}P. Geranylgeranyl diphosphate (GGDP) and solanesyl diphosphate (SDP) showed a triplet signal centered at δ 4.36, with an asymmetric splitting of 6.50, 5.90 Hz and 6.59, 5.86 Hz, respectively. The ^{1}H NMR spectrum of SDP is shown in Fig. 4(b). The chemical shift and splitting of the triplet signals in GGDP and SDP were in good agreement with those observed in the *trans*-1,4-polyisoprene from leaves. This indicates that the triplet signal is assignable to the terminal methylene protons linked-up with a diphosphate ester group [9,10]. The methylene protons of the C-4 carbon of the AX_2M system were found to give a triplet signal due to an additional coupling effect from phosphorus nuclei.

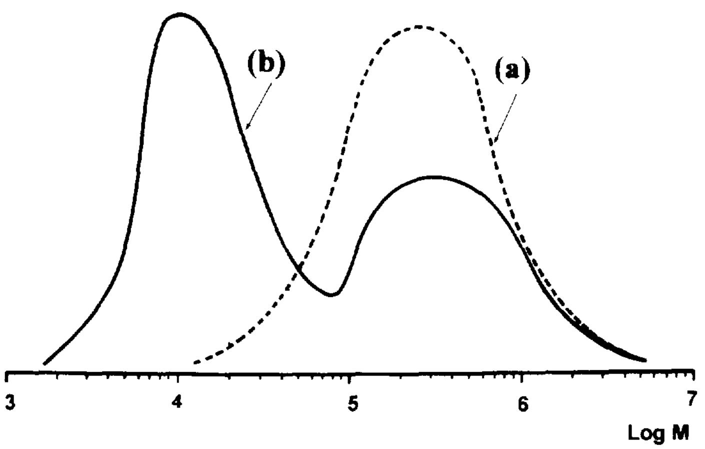


Fig. 2. Molecular-weight distribution of *trans*-polyisoprene from *E. ulmoides* Oliv. extracted from (a) bark and (b) leaves.

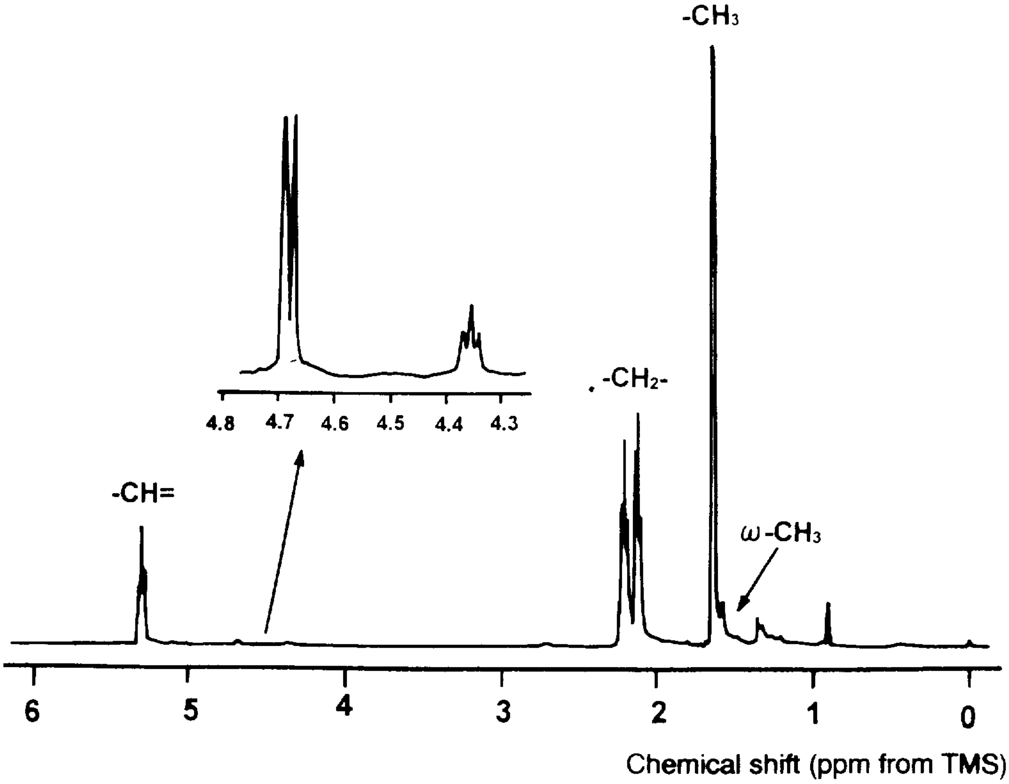


Fig. 3. ^{1}H NMR spectrum of low molecular-weight *trans*-polyisoprene from leaves of *E. ulmoides* Oliv.

The unsymmetrical spacing within each peak in this triplet signal, suggests the occurrence of signals overlapping in a quartet signal to produce the triplet signal.

The presence of a diphosphate terminal-group was further confirmed by treatment of the *trans*-polyisoprene with sodium methoxide. The triplet signal at δ 4.36 and the doublet signal at δ 4.67 completely disappeared, and two new doublet signals at δ 4.12 and 3.99 appeared. These were interpreted as the methyl protons of a primary alcohol ($-CH_2-OH$), and the methine proton of a secondary alcohol ($>CH-OH$), respectively, as shown in Fig. 5(a). As far as we are aware, this is the only instance in which the terminal diphosphate expected from the mechanism of polyisoprene biosynthesis, has been detected.

The relative intensity of the methyl proton signals at δ 1.64, due to the internal *trans*-isoprene, and those in the dimethylallyl-group (Z-CH_3), was estimated to be 68, which is in fair agreement with the average-degree of polymerization of 74, determined by VPO. It is noteworthy that the number of α-terminal groups in the lowest molecular-weight fraction, were 0.64 and 0.28 for the long-chain fatty acid ester and diphosphate terminals, respectively, against the dimethylallyl-group, as determined by the relative intensities of the corresponding signals.

The ^{13}C NMR signals, characteristic of the dimethylallyl-group, were observed at δ 25.64 (Z-C-1 CH_3) and δ 17.66 (E-C-5 CH_3) in the spectrum of the lowest molecular-weight *trans*-polyisoprene (Fig. 6). The average degree of polymerization was estimated to be 107 from the relative intensity between the methyl-carbon signal of the internal *trans*-isoprene units at δ 16.06, and that of the dimethylallyl-group at δ 17.66. The signal of the terminal methylene-carbon (α-C-4) linked-up with the fatty acid ester group was observed at δ 61.16, as in the case of *trans*-polyisoprene from Chicle, Gutta Percha and Balata [5, 9–11]. However, the signal corresponding to the diphosphate terminal was not detected. It is interesting that the terminal C-4 methylene-carbon in long-chain isoprenyl diphosphate also showed no distinct signal [12, 13]. GGDP showed doublet signals centered at δ 61.95 which arose from the methylene-carbon bonded to the diphosphate group, but this signal was not found in SDP (Tamaka, Y., Tangpakdee, J. and Sato, S. unpublished results). In addition, two signals at δ 63.26 and 60.65 due to the C-3 and C-2 carbon atoms in a *trans*-configuration of epoxy-group, respectively, were detected.

After treatment of the *trans*-polyisoprene with sodium methoxide, the signal at δ 59.44 due to the α-C-4 terminated with a primary hydroxyl-group, was detected, as shown in Fig. 7, which was in good agreement with the result found with Chicle [4]. In addition, two signals observed at δ 68.90 and 73.49 were assigned to a C-3 of the secondary alcohol and C-2 of the tertiary alcohol, respectively, which might have arisen by cleavage of epoxide groups.

The other fractions of *trans*-polyisoprene extracted from leaves and bark showed a ^{1}H NMR spectrum similar to that of the lowest molecular-weight one

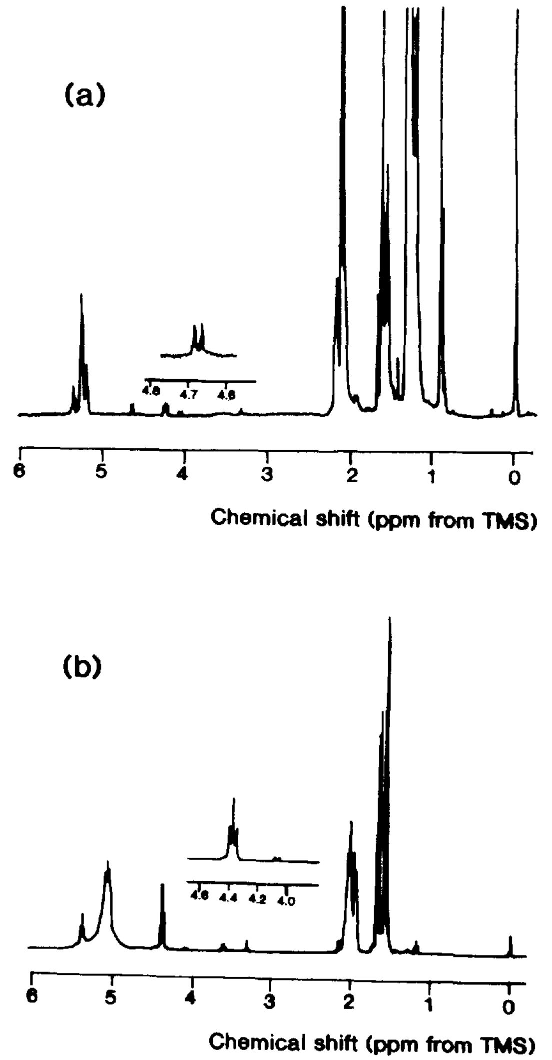


Fig. 4. ^{1}H NMR spectra of (a) solanesyl stearate and (b) solanesyl diphosphate.

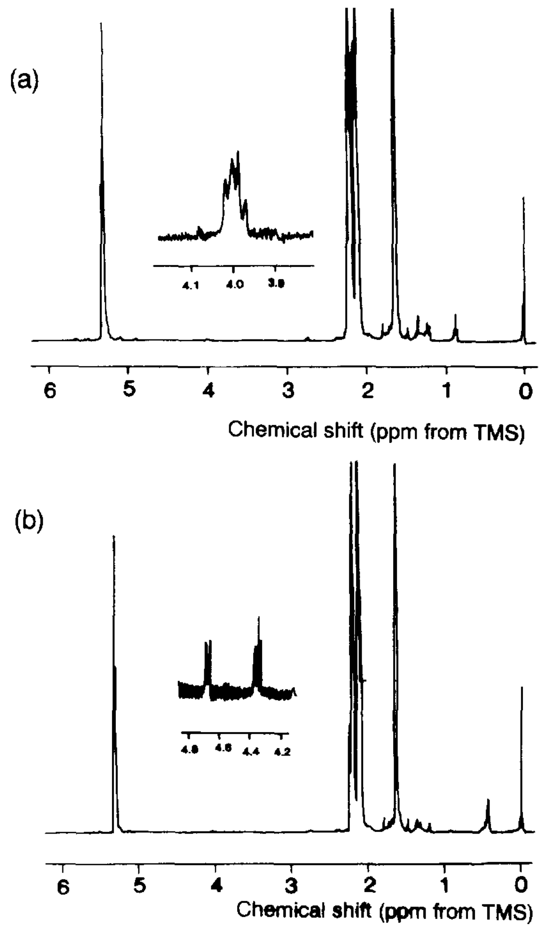


Fig. 5. ^{1}H NMR spectra of (a) transesterified *trans*-polyisoprene from leaves; and (b) bark of *E. ulmoides* Oliv.

(Fig. 5(b)). It is noteworthy that the ratio of the intensities of the signals at δ 4.67 and 4.36 decreased as the molecular weight of the *trans*-polyisoprene increased. In the case of the lowest molecular-weight fraction, the relative intensity of these signals was found to be 3 : 1, while for the highest molecular-weight fraction, it was 1 : 1. If the *trans*-polyisoprene terminating with a long-chain fatty acid ester is considered as dead-polymer and the one carrying a diphosphate terminal group as living-polymer, it is reasonable to suggest that most of the polymer in the lowest molecular-weight fraction would be expected to be dead-polymer.

On the basis of the structural evidence, the mechanism of biosynthesis of *trans*-polyisoprene in *E. ulmoides* Oliv. can be suggested. The initiation of polymerization starts either from DMADP or a *trans*-allylic-diphosphate such as GDP, FDP or GGDP. After successive all-*trans*-addition of IDP, the resulting polyisoprenyl diphosphate is terminated by formation of a fatty acid ester. At present, it is not clear whether esterification occurs after, or during, removal of the diphosphate group.

The characteristic bimodal molecular-weight distribution of the *trans*-polyisoprene from *E. ulmoides* Oliv. leaves, like that of *cis*-polyisoprene from *Hevea* rubber [14], may be interpreted in at least two ways, i.e. the presence of two types of polyprenyltransferases having different specificities or the presence of two sites of polymerization.

Observation by electron microscopy of a green leaf stem showed that *trans*-polyisoprene is contained within laticifers as fibres (Fig. 8). A similar organization was also observed in other parts of leaves and bark. This is a characteristic of *trans*-polyisoprene from *E. ulmoides* Oliv.

EXPERIMENTAL

All *trans*-geranylgeraniol and solanesol were gifts from Dr A. Kageyu, Kuraray, Japan. All other reagents were special grade purchased from Tokyo Kasei Organics Chemical Company (Japan). The diphosphate compounds were prepared according to the procedure of Danilov [12, 13]. The standard method for esterification using stearic chloride was

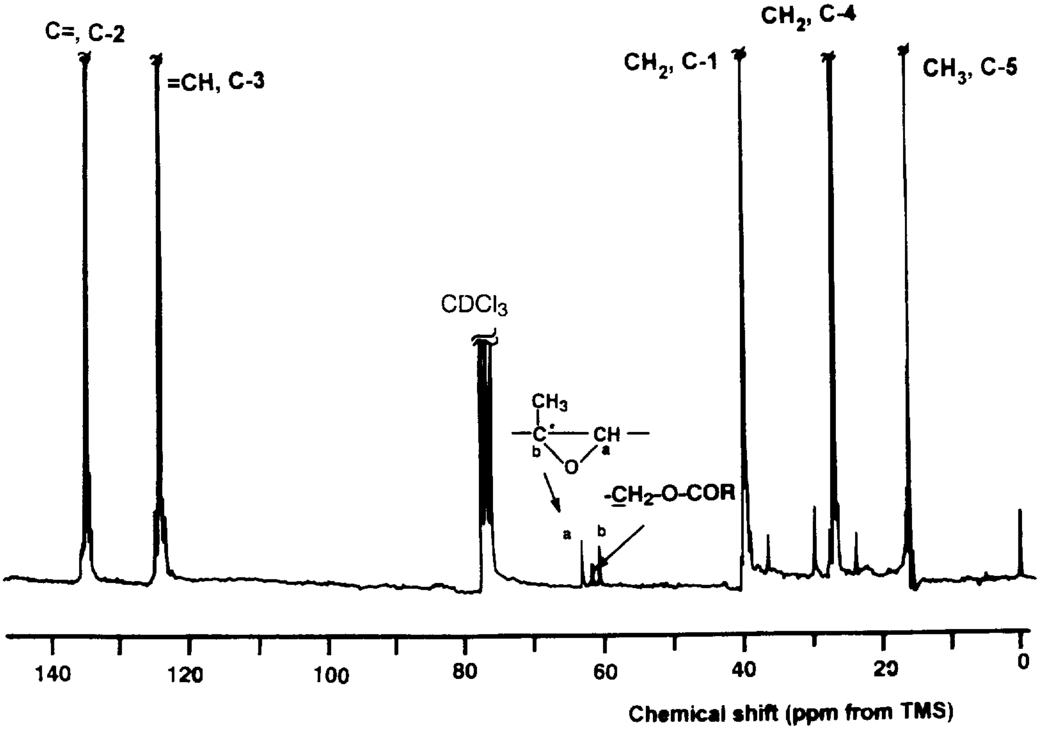


Fig. 6. ^{13}C NMR spectrum of low molecular-weight *trans*-polyisoprene from *E. ulmoides* Oliv. leaves.

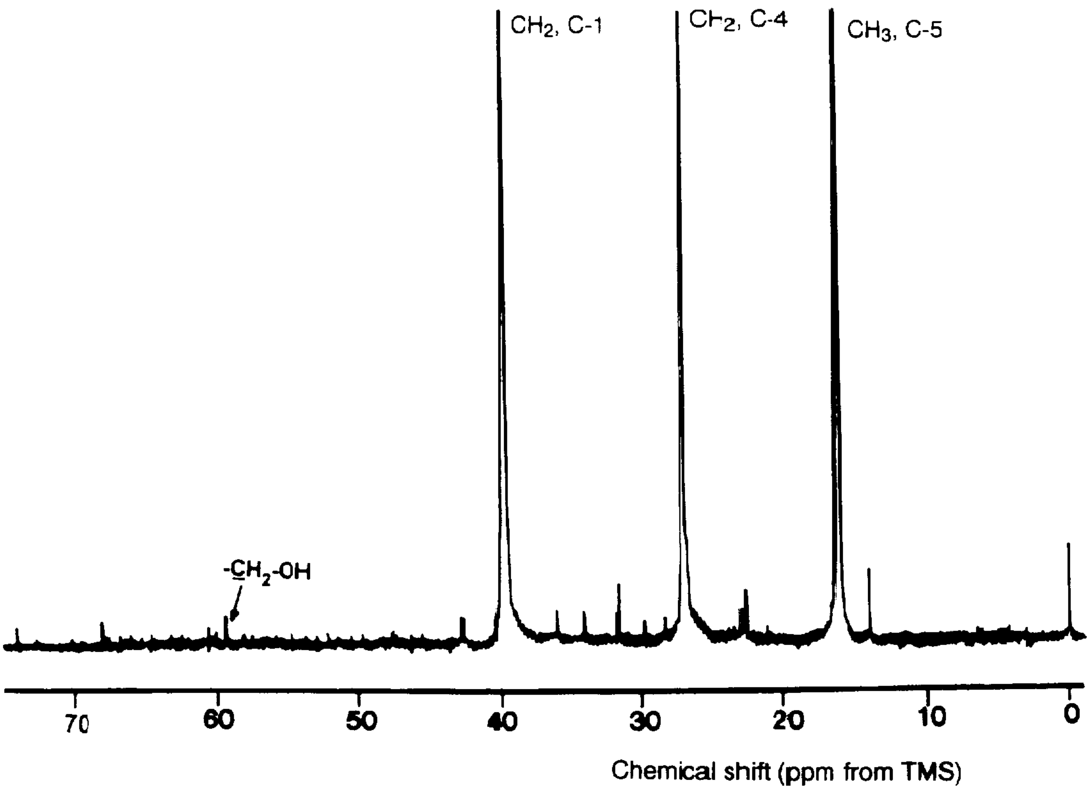


Fig. 7. ^{13}C NMR spectrum of transesterified *trans*-polyisoprene from *E. ulmoides* Oliv. leaves.

Fig. 8. Electron micrograph of green stem of *E. ulmoides* Oliv. leaves (× 100).

used for synthesis solanesyl stearate [15]. Transesterification was carried out by treatment a solution of 1% (w/v) of polymer in toluene with 1 M NaOMe under N_2 in the dark at room temperature for 2 hr [14].

Plant materials. Leaves and bark from a 5-year-old tree of *E. ulmoides* Oliv. growing at Lotte Central Laboratory, Tokyo, were used. The leaves or bark were frozen in liquid nitrogen and crushed in small pieces. EtOH-soluble material was removed by soaking for 60 hr. The residue was extracted in a Soxhlet apparatus with EtOH for 20 hr, followed by hexane for 20 hr under N_2. The resulting polyisoprene was purified by reprecipitation from toluene with MeOH (3 ×), and then recrystallization from a 1 to 2% soln in hot hexane. The yields of *trans*-polyisoprene were 3.8% and 8.7% (w/w) of the dry weight of leaves and bark, respectively. *Trans*-polyisoprene from leaves was fractionated by four successive precipitations from toluene-MeOH in the usual way [16].

GPC, DSC and NMR measurements. GPC was carried out using two columns in series packed with polystyrene-divinylbenzene copolymer gels having exclusion limits of 8×10^6 and 1.4×10^4. The number-average molecular weight measurement was done with a HITACHI-117 Vapor Pressure Osmometer. DSC was done using a SEIKO DSC-220 with heating from $-130°$ to $100°$, and a scanning rate of $10° \min^{-1}$. The ^1H NMR spectra were taken with a JEOL GX-270 spectrometer at 50° in deuterated benzene, with TMS as the internal standard, and a pulse repetition time of 4 sec. The ^{13}C NMR measurements were done with the same instrument at 68.70 MHz at 50° in deuterated chloroform, with a pulse repetition time of 6 sec.

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