J. nat. Rubb. Res., 3(3), 177–183

Structure and Biosynthesis Mechanism of Trans-polyisoprene from Chicle

YASUYUKI TANAKA*, KAZUKI NUNOGAKI*, AKIRA KAGEYU**,
MAKIO MORI* AND YOSHINORI SATO***

The structure of both terminal units and arrangement of isoprene units were determined for trans-polyisoprene isolated from chicle resin by crystallisation in hexane. The trans-polyisoprene showed small ^{13}C-NMR signals characteristic of carbons arisen from the dimethylallyl terminal unit and trans-isoprene unit terminated with hydroxyl group. All of the internal isoprene units were estimated to be in the trans configuration. The number average molecular weight estimated from the relative intensity of both terminal units and internal isoprene units was in good agreement with that of 7.9 × 10^3 determined by GPC-LALLS measurement. GPC-fractionated samples showed the signals from both terminal units with relative intensities directly proportional to the degree of polymerisation. It was concluded that the trans-polyisoprene from chicle is a high molecular weight homologue of Solanesol consisting of dimethylallyl terminal unit, internal trans unit and terminal trans unit substituted for hydroxyl group aligned in that order. The biosynthesis of trans-polyisoprene in chicle was estimated to start from dimethylallyl pyrophosphate by successive addition of isopenetenyl pyrophosphate in trans configuration and to terminate by hydrolysis of the polymer pyrophosphate groups.

Chicle resin is known to contain two types of polyisoprenes in trans and cis configurations. The ratio of trans to cis polymers was reported to be 1 : 1[1], 22 : 78[2], 3 : 7[3] or 1 : 4 in the commercial sample and 1 : 1 in the single tree[4]. Trans-polyisoprene was separated from cis-polyisoprene by precipitation with ethyl acetate from benzene solution or by extraction of cis-polymer with cold hexane[1]. The GPC curves of polyisoprenes from fresh chicle latex and commercial chicle resin showed a typical bimodal molecular weight distribution; the average molecular weights of the trans peak were estimated to be about 10^4 and those of the cis peak[4] to be 1.5 × 10^5. Although a complete separation of both polymers was not reported, these findings suggest that chicle polyisoprene is a mixture of trans- and cis-polyisoprenes having different molecular weights. We have analysed the arrangement of cis and trans isoprene units in cis-trans isomerised polyisoprenes and chicle polyisoprenes by ^{13}C-NMR spectroscopy[5]. The signals characteristic of cis-trans linkages were not detected in trans-polyisoprene separated from commercial chicle resin. However, it was found that trans-polymers separated according to the method of Schlesinger and Leeper were always accompanied by small amounts of isoprene units in cis configuration, estimated to be a contaminant from cis-polyisoprene.

The biosynthesis mechanism of polyisoprene has been studied for cis-polyisoprene from *Hevea brasiliensis* (*Hevea* rubber) and *Parthenium argentatum* (guayule rubber). The steps of formation of acetyl-CoA to isopentenyl pyrophosphate *via* mevalonate were clarified by biochemical studies using mainly tracer techniques. The chain extension step was established to

*Department of Material Systems Engineering, Faculty of Technology, Tokyo University of Agriculture and Technology, Koganei, Tokyo 184, Japan

**Present Address: Central Research Laboratory, Kuraray Co. Ltd., Sakazu, Kurashiki, Okayama 710, Japan

***Lotte Central Laboratory Co. Ltd., Numakage, Urawa, Saitama 336, Japan

occur by successive additions of isopentenyl pyrophosphate on the existing polymer pyrophosphate in cis configuration[6,7]. The initial step of rubber formation was proposed to start from dimethylallyl pyrophosphate by analogy with the biosynthesis of trans-terpenoid compounds[8]. However, there was no direct evidence to prove the initiation mechanism. No work appears to have been carried out on the mechanism terminating the polymer pyrophosphate. On the other hand, the biosynthesis of trans-polyisoprene has not been studied.

We have established a new method to characterise the arrangement of isoprene units from both terminal units in acyclic isoprenoid compounds by using ^{13}C-NMR spectroscopy[9,10]. The ^{13}C-NMR method was applied to the structural characterisation of cis-polyisoprene from the leaves of goldenrod[11] and sunflower, and from guayule and *Hevea* rubbers[12,13]. It has been found that cis-polyisoprenes isolated from the leaves consist of dimethylallyl terminal unit, three trans units and a long sequence of cis units terminated with hydroxyl group aligned in that order[14]. These findings demonstrate that the formation of cis-polyisoprene starts from trans, trans, trans-geranylgeranyl pyrophosphate as a direct primer in the case of the rubber isolated from the leaves.

This paper reports the structural characterisation of trans-polyisoprene from chicle resin by using the ^{13}C-NMR method. The biosynthesis mechanism is estimated on the basis of the chemical structure of both terminal units and the alignment of isoprene units.

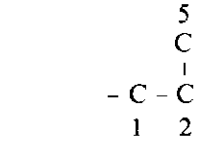
EXPERIMENTAL

The polyisoprene fraction was isolated from commercially obtained chicle resin by Soxhlet extraction with acetone for 24 h under nitrogen atmosphere. The yield of the polymer fraction was 29%. Trans-polyisoprene was isolated ten times by recrystallisation from 5% to 2% weight/volume hexane solution at 25°C to 5°C. GPC-LALLS measurements were made with a JASCO TRIROTAR-II high pressure pump and a TOSOH LS-8000 low-angle laser light scattering photometer (LALLS) equipped with TOSOH RI-8011 detector. Analytical GPC measurements were made using two columns (10.0 mm internal diameter × 500 mm) packed with polystyrene gel having an exclusion limit of 2×10^6 and 2×10^5 in series. The GPC fractionation was carried out by using a preparative column (21.2 mm internal diameter × 500 mm) packed with polystyrene gel having an exclusion limit of 5×10^6. The molecular weights of trans- and cis-polyisoprenes were determined by GPC-LALLS measurements. The ^{13}C-NMR spectra were obtained at 50.1 MHz with a JEOL FX-200 in $CDCl_3$ solution (5%–12% weight/volume) at 50°C with multiple scans at a pulse repetition time of 7 s for a 45° pulse. Chemical shifts were referred to tetramethylsilane as an internal standard. The accuracy of the chemical shifts was ± 0.01 p.p.m.

RESULTS AND DISCUSSION

Typical bimodal GPC and LALLS curves were observed for chicle polyisoprene separated from acetone-soluble resin fraction as shown in *Figure 1*. The molecular weight of cis-polyisoprene was estimated to be $\bar{M}n = 1.4 \times 10^5$ with polydispersity of $\bar{M}w/\bar{M}n = 1.3$. The ratio of cis to trans peaks was found to be 25:75. As shown in *Figure 2*, trans-polyisoprene isolated by repeated recrystallisation showed a similar molecular weight distribution as the low molecular weight peak in *Figure 1*. The molecular weight was estimated to be $\bar{M}n = 7.9 \times 10^3$ with polydispersity of $\bar{M}w/\bar{M}n = 1.2$.

The ^{13}C-NMR spectrum of trans-polyisoprene showed small signals in addition to the five major signals corresponding to carbon atoms in trans units as shown in *Figure 3*. These signals were assigned to the carbons of the internal and both terminal units as listed in *Table 1*. Here, the carbon atoms in isoprene units including both the terminal units are designated as follows:



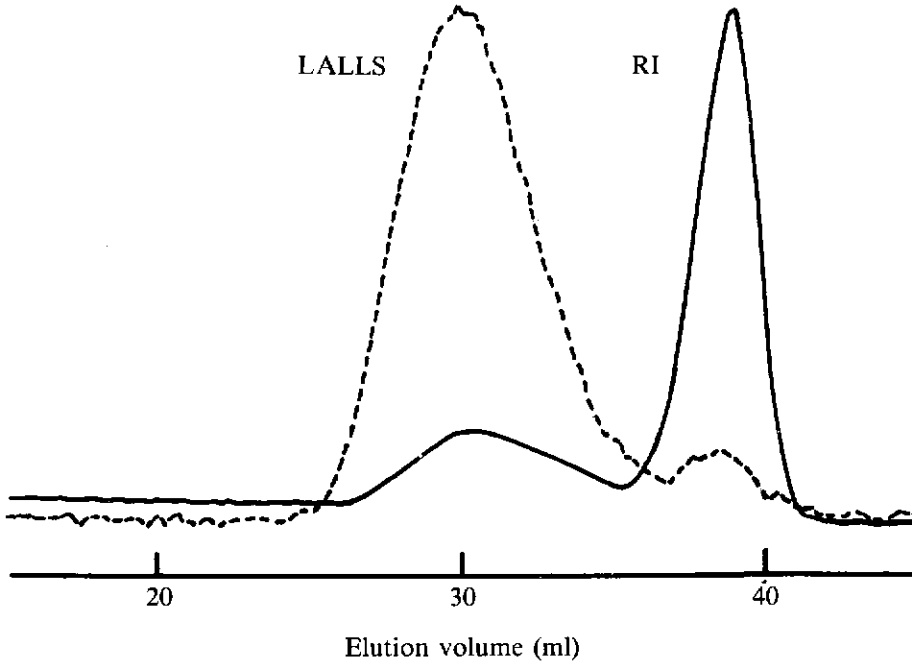


Figure 1. GPC-LALLS curve for polyisoprene from chicle (LALLS: low-angle laser light scattering detector; RI: refractive index detector).

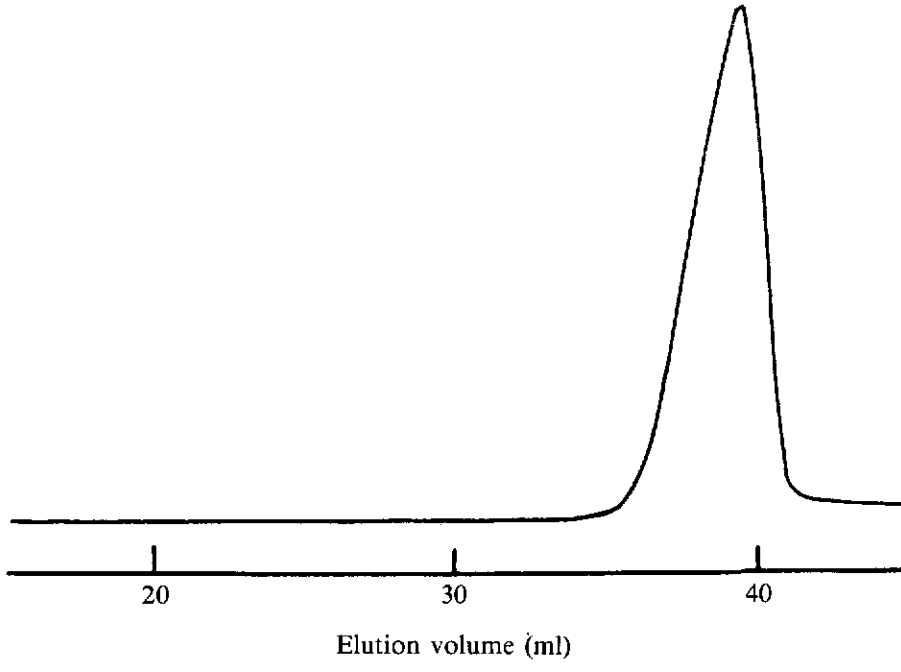


Figure 2. GPC curve for trans-polyisoprene.

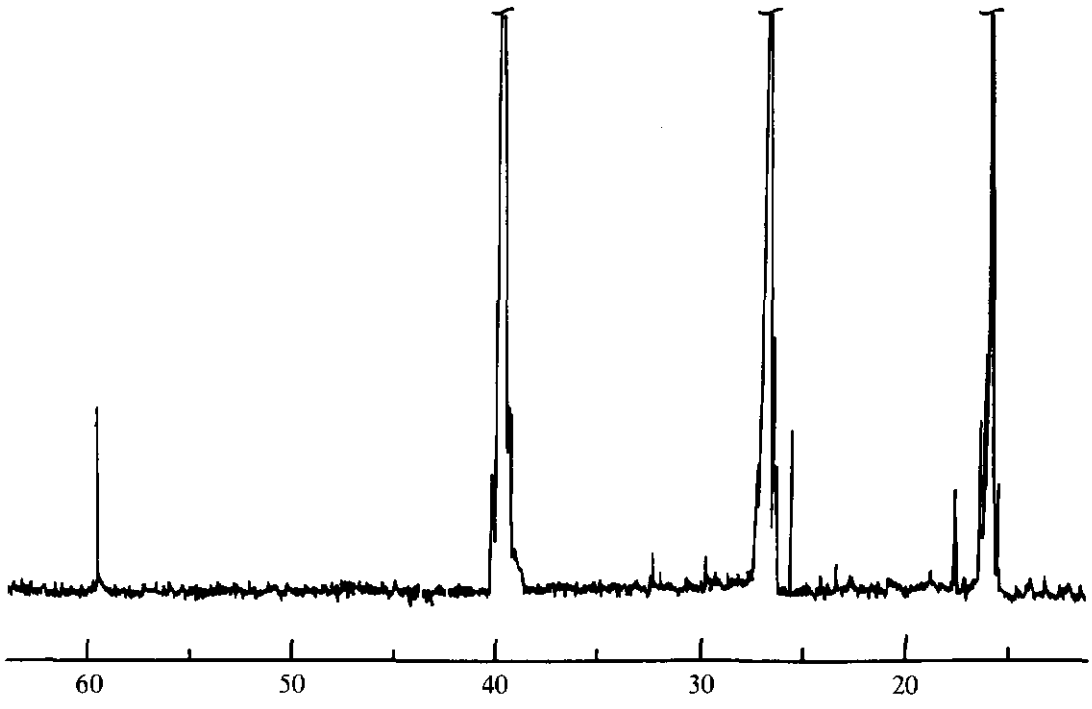


Figure 3. ^{13}C-NMR spectrum of trans-polyisoprene.

TABLE 1. ASSIGNMENT OF ^{13}C-NMR SIGNALS IN TRANS-POLYISOPRENE

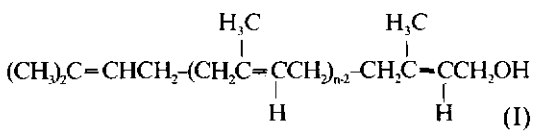
Chemical shift (p.p.m.)	Assignment		
139.72	α	C-2	=C-
134.91	trans	C-2	=C-
131.10	ω	C-2	=C-
124.37	trans	C-3	=CH
59.43	trans (α)	C-4	CH_2
39.77	trans	C-1	CH_2
26.83	trans	C-4	CH_2
25.63	ω	C-1	CH_3
17.65	ω	C-5	CH_3
16.04	trans	C-5	CH_3

The small signal resonated at 59.43 p.p.m. is assigned to the C-4 methylene carbon of the terminal trans isoprene unit linked to the hydroxyl group by comparison of the chemical shifts of model compounds[10,15]. The configuration of this terminal isoprene unit, which is designated as α-terminal, is assignable to trans; the corresponding carbon atom in the terminal cis isoprene unit is expected to resonate around 59.0 p.p.m. The small signals at 25.63 p.p.m. and 17.65 p.p.m. can be assigned to the methyl carbons of the dimethylallyl terminal unit, which is designated as ω-terminal, in *(Z)* and *(E)* configurations, respectively.

The configuration of the isoprene unit linked to the ω-terminal unit can be determined from the chemical shift of the C-2 olefinic carbon in ω-terminal unit, that is, the carbon in the ω-trans linkage is expected to resonate around 131.1 p.p.m. and the corresponding carbon in the ω-cis linkage at 131.5-131.6 p.p.m.[15]. A small signal observed at 131.10 p.p.m. in *Figure 3* supports the assumption.

In *Figure 3*, very small signals were detected at 32.28 p.p.m. and 23.39 p.p.m., which are assignable to the C-1 methylene and C-5 methyl carbons in cis isoprene units, respectively[5,10]. The relative intensity of these signals decreased by purification to as low as 0.16% against the corresponding signals of trans units after ten times recrystallisation in hexane. The chemical shift of the C-1 methylene carbon signal showed that the cis units are in the cis-cis linkage[10] showing that these signals are derived from residual cis-polyisoprene as a mixture.

These findings demonstrate that trans-polyisoprene from chicle consists of a dimethyl-allyl terminal unit and a long sequence of trans units terminated with a hydroxyl group as shown below:



(I)

This indicates that trans-polyisoprene from chicle is a high molecular weight homologue of Solanesol, which is a nonamer ($n=9$ in I) widely distributed in the plant kingdom.

Trans-polyisoprene from chicle was fractionated into three fractions by GPC. The relative intensities of the aliphatic carbon signals were listed in *Table 2* together with the degree of polymerisation of these samples. Here, the Nuclear Overhauser Effect factors (NOE) and the spin-lattice relaxation times (T_1) were estimated for three major signals of the original sample and for Solanesol as a model compound. A fairly good agreement was observed between the degree of polymerisation and intensity ratio of C-1 and C-4 methylene carbons against α C-4 methylene carbon. The relative intensity of the ω C-1 methyl to α C-4 methylene carbons was close to one for all the samples. These four signals have almost full NOE and T_1 values short enough to permit quantitative treatment. Consequently, it may be possible to conclude that trans-polyisoprene from chicle is a linear polymer having both terminal units as shown in the structure (I).

On the basis of the structural evidence it may be possible to estimate the biosynthesis mechanism of trans-polyisoprene from chicle as illustrated in *Figure 4*. The initiating species is estimated to be dimethylallyl pyrophosphate or trans-isoprenoid pyrophosphate such as farnesyl or geranylgeranyl pyrophosphate. The polymer chains are formed by successive addition of isopentenyl pyrophosphate in trans configuration and terminated by simple hydrolysis of the pyrophosphate terminal group as in the case of goldenrod rubber[11].

TABLE 2. RELATIVE INTENSITY OF ALIPHATIC CARBON SIGNALS

Chemical shift (p.p.m.)	Assignment	Relative intensity				NOE	T_1 (s)
		Original sample	Fractionated samples				
			Fr. 1	Fr. 2	Fr. 3		
59.41	α C-4	1	1	1	1	— (2.76)	— (2.5)
39.78	trans C-1	107	192	100	94	3.28 (2.72)	0.5 (0.8)
26.78	trans C-4	106	184	107	83	2.90 (2.82)	0.5 (1.2)
25.67	ω C-1	0.95	1.1	1.1	1.1	— (2.51)	— (3.8)
17.67	ω C-5	0.60	0.66	0.72	0.85	— (1.63)	— (7.0)
16.04	trans C-5	96	152	84	78	2.31 (1.78)	4.7 (4.1)
DP[a]		110	190	100	70		

Figures within brackets are determined for Solanesol as a model compound.

[a]Degree of polymerisation by GPC

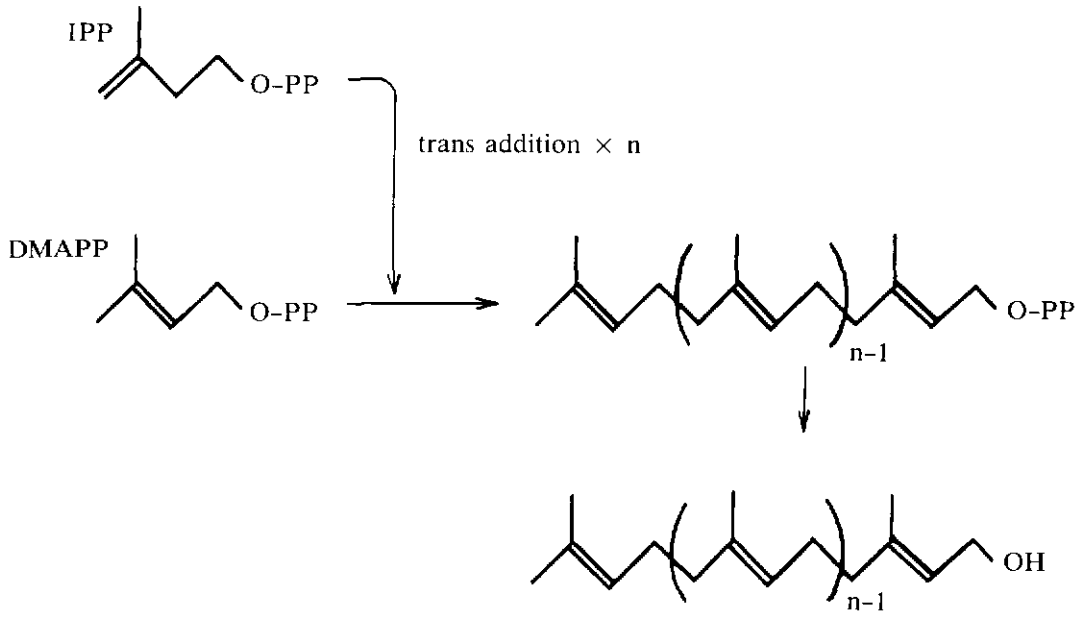


Figure 4. Biosynthesis mechanism of trans-polyisoprene in chicle.

It is worth noting that this type with both terminal units was not detected in cis-polyisoprenes occurring as latex, although the fundamental structure of these rubbers is the same as that of rubbers from goldenrod and sunflower, consisting of three trans units and a long sequence of cis units[14]. It can be speculated that some special species initiate the cis polymerisation or some selective reactions occur on both terminal units after polymerisation for the absence of dimethylallyl units and hydroxyl terminal units in cis-polyisoprene occurring in latex. Despite chicle polyisoprene occurs as latex, trans-polyisoprene from chicle shows the same terminal units as goldenrod rubber. The biochemical characteristics of the formation of trans-polyisoprene in chicle will be clarified by the comparison of the detailed structure with that from gutta percha and balata, which will be reported in a subsequent paper.

This work has been supported in part by a grant-in-aid from the Ministry of Education, Science and Culture, Japan; Grant No. 62470102.

REFERENCES

1. SCHLESINGER, W. AND LEEPER, H.M. (1951) Chicle cis- and trans-Polyisoprenes from a Single Plant Species. *Ind. Engng Chem.*, **43**, 398.

2. STAVELY, F.W. BIDDISON, P.H., FORSTER, M.J., DAWSON, H.G. AND BINDER, J.L. (1961) The Structure of Various Natural Rubbers. *Rubb. Chem. Technol.*, **34**, 423.

3. ARCHER, B.L. AND AUDLEY, B.G. (1973) Rubber, Gutta Percha and Chicle. *Phytochemistry Vol. 2, (Miller, L.P., ed.)*, pp. 310–343. New York: Van Nostrand Reinhold.

4. HAGER, T., MACARTHUR, A., MCINTYRE, D. AND SEEGER, R. (1979) Chemistry and Structure of Natural Rubbers. *Rubb. Chem. Technol.*, **52**, 693.

5. TANAKA, Y. AND SATO, H., (1976) Sequence Distribution of cis-1,4- and trans-1,4-Units in Polyisoprenes. *Polymer*, **17**, 113.

6. LYNEN, F. AND HENNING, U. (1960) Über den biologischen Weg zum Naturkautschuk. *Angew. Chem.*, **72**, 820.

7. ARCHER, B.L., AYREY, G., COCKBAIN, E.G. AND MCSWEENEY, G.P. (1961) Incorporation of (1-^{14}C) Isopentenyl Pyrophosphate into Polyisoprene. *Nature*, **189**, 663.

8. LYNEN, F., EGGERER, H., HENNING, U. AND KESSEL, I. (1958) Farnesyl-pyrophosphat und 3-Methyl-3-butenyl-l-pyrophosphat, die biologischen Vorstufen des Squalens. Zur Biosynthese der Terpene, III. *Angew. Chem.*, **70**, 738.

9. TANAKA, Y. AND TAKAGI, M. (1979) Structural Characterization of Ficaprenol-11 by ^{13}C-Nuclear Magnetic Resonance. *Biochem. J.*, **183**, 163.

10. TANAKA, Y., SATO, H. AND KAGEYU, A. (1982) Structural Characterization of Polyprenols by ^{13}C-NMR Spectroscopy: Signal Assignments of Polyprenol Homologues. *Polymer*, **23**, 1087.

11. TANAKA, Y., SATO, H. AND KAGEYU, A. (1983) Structure and Biosynthesis Mechanism of Natural cis-Polyisoprene from Goldenrod. *Rubb. Chem. Technol.*, **56**, 299.

12. TANAKA, Y. (1985) Structural Characterization of cis-Polyisoprene from Sunflower, *Hevea* and Guayule. *Proc. Int. Rubb. Conf. Kuala Lumpur 1985*, **2**, 73.

13. TANAKA, Y., HIGUCHI, K. AND MAEDA, M. (1986) The Chemical Structure of Naturally Occurring cis-Polyisoprenes isolated from Sunflower, *Hevea* and Guayule Determined by NMR Spectroscopy. *Proc. Wkshop on Biochemistry and Regulation of cis-Polyisoprene in Plants (Benedict, C.R., ed.)*, 12.

14. TANAKA, Y. (1987) The Structure and Biosynthesis Mechanism of Naturally Occurring Polyisoprenes. *Proc. Int. Rubb. Conf. Harrogate, England 1987*, **4A**, 1.

15. TANAKA, Y. (1984) *NMR and Macromolecules, (Randall, J.C., ed.)*, 233. American Chemical Society.