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LONG-CHAIN POLYPRENOLS AND RUBBER IN YOUNG LEAVES OF *HEVEA BRASILIENSIS*†

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Key Word Index—*Hevea brasiliensis*; Euphorbiaceae; long-chain polyprenols; rubber biosynthesis; terminal groups.

Abstract—Long-chain polyprenols consisting of 10–70 *cis*-isoprene units are present in the latex of nascent leaves of young *Hevea* shoots. A continuous chain-length distribution was observed in these polyprenols, as commonly found in polyprenols isolated from other higher plants. ^{13}C NMR analysis showed that they contained no ω-dimethylallyl-group at the initiating end, as is the case with the high and low M_r rubber from *Hevea*. This finding supports the assumption that the initiating species in the biosynthesis of rubber and polyprenols in *Hevea* is not *trans-trans*-farnesyl-diphosphate (*t,t*-FDP) but an FDP, in which the dimethylallyl-group is modified. Alternatively, the initiator is *t,t*-FDP and the dimethylallyl-group is changed after polymerization. © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

There are two main types of polyprenols based on their geometric isomerism, viz, di-*trans* poly-*cis* prenols and tri-*trans* poly-*cis* prenols, as identified by NMR spectroscopy [1]. It is assumed that they are formed from di-*trans*-farnesyl diphosphate and tri-*trans*-geranylgeranyl diphosphate, respectively, by the action of *cis*-prenyltransferases. Analogous enzymes are presumed to have a similar function in rubber formation.

That short- and long-chain *cis*-polyprenols accumulate in many plant species has been observed over several decades [2]. Short-chain polyprenols composed of 11–12 isoprene residues were found in leaves of some angiosperms and long-chain polyprenols have been isolated from green leaves of several angiosperms and gymnosperms [3], including those in the wood of *Betula verrucosa* [4]. These polyprenols have M_rs lower than 2000. Recently, the longest polyprenols, known so far, comprising ca 100 isoprene units were isolated from *Potentilla aurea* and their presence in several species of the *Rosaceae* was reported [5]. However, until the present work, long-chain polyprenols have not been reported to occur in *Hevea*.

It is well known that *cis*-polyisoprenes obtained from *H. brasiliensis* and *Parthenium argentatum* consist of more than 5000 isoprene units and that their weight average M_r is higher than one million. In contrast, there are some species producing *cis*-polyisoprenes of M_r less than 100000, e.g. *Pyla lanceolata*, *Eupatorium altissimum* [6], *Ambrosia trifida* [7] and *Solidago altissima* [8]. The structure of a relatively low M_r rubber from sunflower leaves, was shown to be built from 320–360 internal *cis*-isoprene units, one dimethylallyl group and two to three *trans*-isoprene units [8].

The present paper reports on the presence and characterization of long-chain polyprenols and short-chain rubber in the latex from nascent leaves of young *Hevea* shoots. These materials can be considered to be intermediate in length between short-chain oligoprenols and high M_r rubber. The structural findings are of relevance to the mechanism of initiation of rubber formation in *Hevea*.

RESULTS AND DISCUSSION

Figure 1 shows the M_r distribution (MWD) of rubber obtained from the latex exuded from nascent leaves of *Hevea* young shoots after it had been saponified. It clearly shows an abundance of rubber with a M_r peak centred at 1.2×10^5. This MWD is similar to those observed for 7-month-old *Hevea* seedling trees [9]. There are three additional peaks, the first, centred at 3.2×10^6 corresponds to high M_r rubber, and the

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[†] Part 2 in the series "Initiation of rubber biosynthesis in *Hevea brasiliensis*". For Part 1 (see Ref. [14].

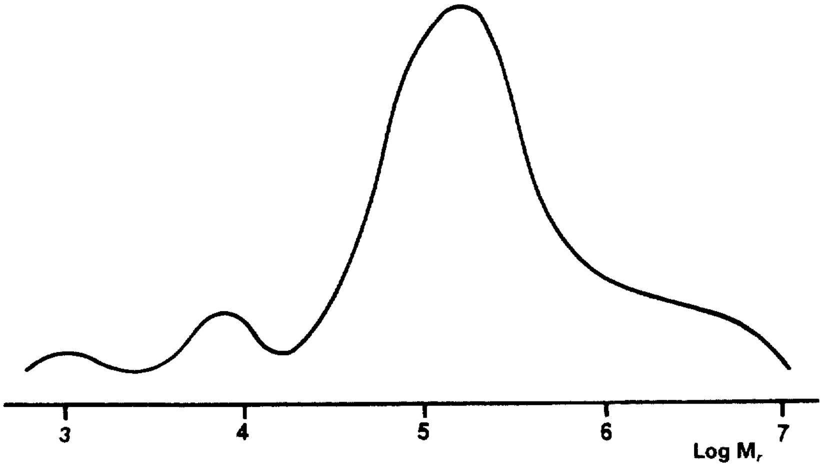

Fig. 1. M_r distribution after saponification of rubber isolated from nascent leaves on young *Hevea* shoots.

other two, centred around 10^4 and 10^3, arise from low M_r rubber and/or long-chain polyprenols. Such low M_r peaks are characteristic of rubber from the latex of nascent leaves of young shoots. Rubbers obtained from *Hevea* seedlings or mature trees show a characteristic bimodal or skewed unimodal MWD [11].

The unsaponified rubber was separated into four fractions by gel permeation chromatography (GPC). The first corresponds to rubber having a M_r higher than 2.0×10^4, the second to rubber having M_rs between 5.0×10^3 and 2.0×10^4, and the third to rubber of M_r lower than 5.0×10^3. The last fraction contained no isoprenoid compounds. The chain-length distributions of the second and third fractions were further analyzed by HPLC (Figs 2a and 2b). The third fraction consists of a series of polyisoprene homologues from 10 to ca 70 isoprene units, with a peak-top around 32, confirming that this fraction corresponds to the last peak in Fig. 1. The chain-length was estimated using a calibration curve obtained from polyprenols extracted from needles of *Pinus thunbergii* [10], indicating that the third fraction contains long-chain polyprenols. It is noteworthy that the chain-length distribution of the polyprenols having less than 20 isoprene units, showed clear splittings for each peak. These are probably due to differences in the terminal groups of the polyprenols, that is, free alcohol and alcohol esterified with fatty acid(s) (polyprenyl esters). As expected, these splittings disappeared on extensive saponification [12,13]. This

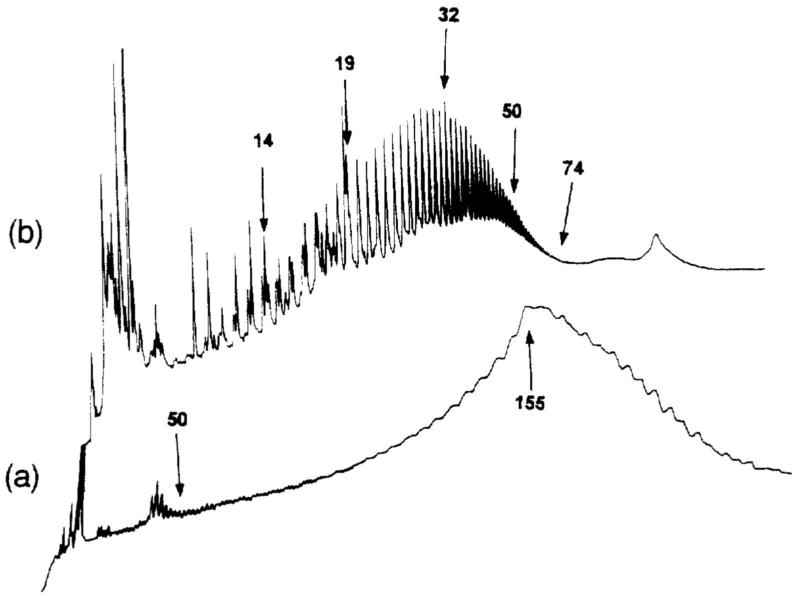

Fig. 2. HPLC profiles of GPC fractions of rubber isolated from nascent leaves on young *Hevea* shoots. (a) fraction 2; (b) fraction 3.

behaviour in chain-length distribution has been observed previously in polyprenols isolated from other species [11].

The chain-length distribution of *cis*-1,4-polyisoprene of more than 50 isoprene units is shown in Fig. 2a. A small amount of residual long-chain polyprenols between nine and 32 isoprene units can be clearly observed in this chromatogram. Although the resolution of this chromatogram was not good, the peak-top of this distribution corresponds to ca 150 *cis*-isoprene units (M_r 1.0×10^4), as calibrated with synthetic *cis*-1,4-polyisoprene. Thus, the second fraction can be related to the small peak due to low M_r rubber in Fig. 1. It is interesting to note that there is a periodic repetition of peaks corresponding to about five isoprene units in this chromatogram. On the other hand, a simple monotonous chain-length distribution was commonly observed for synthetic *cis*-1,4-polyisoprene, suggesting that a characteristic periodic distributions (Fig. 2) truly exist and, presumably, reflect some aspect of the biosynthetic mechanism. The low M_r rubber extracted from *Lactarius volemus* also showed a similar periodic repetition of five or six isoprene units [13].

The HPLC analysis clearly suggests that leaves from young *Hevea* shoots produce long-chain polyprenols as commonly observed in other species, such as *Potentilla* [12], as well as low M_r rubber, as found in the sunflower [9]. This is the first time that long-chain polyprenols, together with high and low M_r rubbers, have been detected in *Hevea*.

The rubbers obtained from mature leaves, freshly tapped latex, a seedling tree, or commercial high-ammonia latex, were shown to contain no polyprenols. Thus, the long-chain polyprenols are present only in the latex from the nascent leaves of young *Hevea* shoots. Recently, we have observed a similar chain-length distribution in rubber extracted from *Lactarius* mushrooms [13]. It showed the presence of at least two families of long-chain polyprenols as well as a periodical repetition in the MWD of the rubber. Based on these findings, it may be suggested that the polyprenols as long as 70 isoprene units are synthesized by polyprenyl-transferases.

Figures 3a–c show the ^{13}C NMR spectra of low M_r rubbers from the second and third fractions, and polyprenol-16, respectively. The average degrees of polymerization of the second and third fractions were estimated to be 194 and 48, respectively, by comparing the ratio of *cis*- and *trans*-isoprene units in the ^{13}C NMR spectrum, on the assumption that there were two *trans*-isoprene units per chain. Figure 3a shows clearly the presence of the characteristic signals for C-1 methylene and C-5 methyl carbon atoms in the *trans*-isoprene units at δ 39.8 and 16.0. Figure 3b shows splittings resonating at δ 39.8 and 39.8, as observed for polyprenol-16, relating to the triad sequences, dimethylallyl-*trans*-*trans* and *trans*-*trans*-*cis*-arrangements, respectively [8]. This is evidence that the third fraction (long-chain polyprenols) in *Hevea* rubber also has two *trans*-isoprene units at the initiating end. However, the methyl-carbon in the dimethylallyl-group, expected to resonate at δ 17.6, was not observed in both the ^{13}C NMR spectra of low M_r, rubbers and long-chain polyprenols. This is in accordance with previous observations on various *Hevea* rubbers, e.g. from freshly tapped latex, seedling rubbers [9] and commercial rubbers. This finding implies that the long-chain polyprenols in latex from nascent leaves have a similar structure to the high and low M_r rubbers. This if further evidence indicating that the initiating species for rubber formation in *Hevea* is not ω-*t*-*t*-FDP but a FDP modified at the dimethylallyl-group. However, it is apparent, that the absence of the dimethylallyl-group could also result from modification after polymerization [14]. As to nature of the initiating terminal, other structural evidence suggests the linkage or association with proteins, which have an important role in forming branch-points in *Hevea* rubber. However, the details of this putative structure remained unresolved at this stage.

EXPERIMENTAL

Plant material

The nascent leaves of young shoots growing on the stump of an old *Hevea* tree, at the rubber plantations of the Rubber Research Institute of Thailand, Hat-Yai, Thailand, were used for this study. Latex exuded from nascent leaves, by cutting with a blade, was immediately dipped and extracted with toluene–hexane (1:1). The dry rubber content of the latex was ca 15%. The extract was conced to small vol. in a rotary evaporator at 40° and ppted with MeOH, followed by reprecipitation of the rubber in toluene containing EtoH (×3). The purified rubber (50 mg) (0.5%, w/v) in toluene–hexane soln (1:2) was saponified in 25 ml KOH (EtOH–H_2O, 5:1, 15% KOH) and 3 ml methanolic pyrogallol (5%, w/v), at 70°, in the dark under N_2, for 1 h. The hot saponified mixt. was then filtered and washed with H_2O until the pyrogallol was removed, followed by washing with hot H_2O (×5).

Polyprenols were extracted from needles of *Pinus thunbergii* and confirmed that their structure and MWD was as expected [10]. Synthetic *cis*-polyisoprene rubber (LIR 30) was supplied by Dr A. Kageyu of the Kuraray Co. and purified by reprecipitation of rubber in toluene containing MeOH (×3).

Molecular weight and NMR analyses

The purified rubber was separated into four frns by GPC using two columns of styrene-divinylbenzene copolymers, with THF as eluent. These frns were dissolved in hexane and washed with hot H_2O. The second and third frns were subjected to analysis on a commercial ODS reverse-phase column (600 × 10.7 mm) with UV detection at 210 nm. A programmed gradient flow was applied by starting with 2-PrOH–

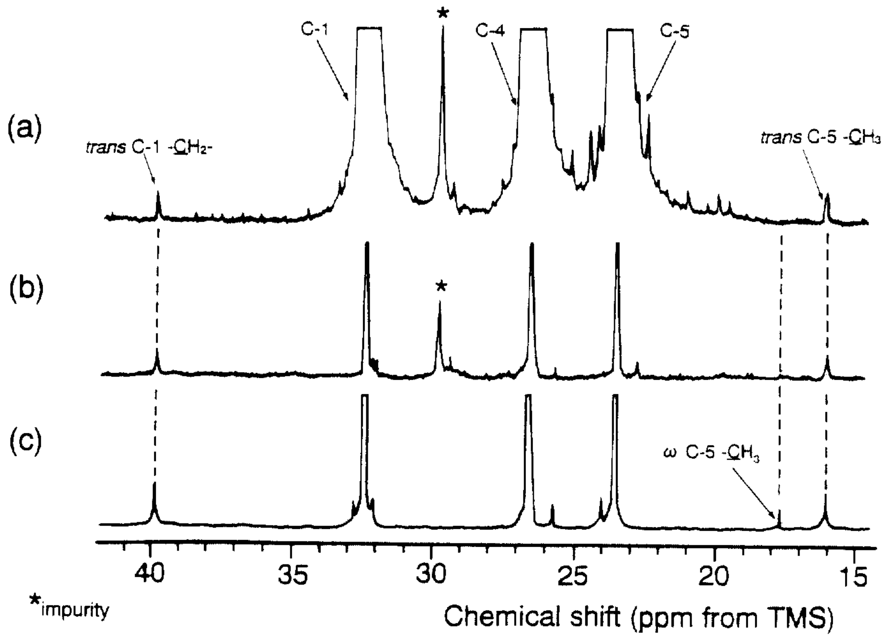

Fig. 3. ^{13}C NMR spectra of fractions 2 and 3 (Fig. 2) and (c) polyprenol-16 (ω-t_2-c_{13}-OH).

MeOH–H_2O (8:12:1) in pump system A and hexane–2-PrOH (7:3) in pump system B (flow rate 0.5 ml min^{-1}) [12].

^{13}C NMR was recorded in $CDCl_3$ at 50°, with TMS as int. standard; the pulse interval was 7 s and 10 000 scans were run.

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REFERENCES

1. Tanaka, Y. and Takagi, M., *Biochemical Journal*, 1979, **183**, 163.
2. Welburn, A. R., Stevenson, J., Hemming, F. W. and Morton, R. A., *Biochemical Journal*, 1967, **102**, 313.
3. Zinkel, D. F. and Evans, B. B., *Phytochemistry*, 1972, **11**, 3387.
4. Lindgren, B. O., *Acta Chemical Scandinavia*, 1965, **19**, 1317.
5. Swieezewska, E. and Chojnacki, T., *Phytochemistry*, 1991, **30**, 267.
6. Buchanan, R. A. and Duke, J. A. N., in *CRC Handbook of Biosolar Resources*, *Vol. II*, ed. T. A. McClure and E. S. Lipinski. CRC Press Inc., Boca Raton, 1979, p.157.
7. Swanson, C. L., Buchanan, R. A. and Otey, F. H., *Journal of Polymer Science*, 1979, **23**, 743.
8. Tanaka, Y., in *Methods in Plant Biochemistry*, *Vol. 7 Terpenoids*, ed. P. M. Dey and J. B. Harborne. Academic Press, London, 1991, p. 519.
9. Tangpakdee, J., Tanaka, Y., Witisuwannakul, R. and Chareonthiphakorn, N., *Phytochemistry*, 1996, **42**, 353.
10. Ibata, K., Mizumo, M., Tanaka, Y. and Kageyu, A., *Phytochemistry*, 1984, **23**, 783.
11. Swiezewska, E., Sasak, W., Mankowski, T., Jankowski, W., Vogtman, T., Krajewska, I., Hertel, J., Skoczylas, E. and Chojnacki, T., *Acta Biochimica Polonica*, 1994, **41**, 221.
12. Swiesewska, E. and Chojnacki, T., *Acta Biochimica Polonica*, 1989, **36**, 143.
13. Ohya, N., Takizawa, J., Kawahara, S. and Tanaka, Y., *Phytochemistry*, in press.
14. Tanaka, Y., Eng, A. H., Ohya, N., Nishiyama, N. Tangpakdee, J., Kawahara, S. and Wititsuwannakul, R., *Phytochemistry*, 1996, **41**, 1501.
15. Brown, M. J., Milano, P. D., Lever, D. C., Epstein, W. W. and Poulter, C. D., *Journal of American Chemical Society*, 1991, **113**, 3176.