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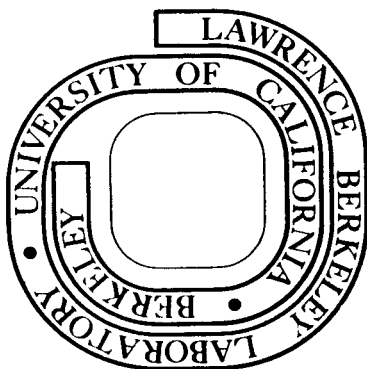
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LIPIDS OF HEVEA BRASILIENSIS AND EUPHORBIA COERULESCENS¹

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Key Words: *Hevea brasiliensis*; *Euphorbia coerulescens*; Euphorbiaceae;
lipids; sterols; hydrocarbons.

Abstract

The low molecular weight lipids identified in the latices of Hevea brasiliensis and Euphorbia coerulescens were as follows: 2-methylcyclobutanone; 2-methyl-2-hydroxycyclobutanone; 2-methylcyclobutanol; euphol; euphorbol; and tirucallol.

Introduction

As a result of decreasing supplies of fossil hydrocarbons it has become necessary to re-examine other sources of raw materials for possible conversion into hydrocarbons.¹ It is well known that sources such as sugar-cane and rubber trees (Hevea) can produce energy-rich materials under suitable conditions.² Carbohydrate from sugar-cane and other plants is converted to alcohols by fermentation and subsequently to olefins by dehydration. The same olefins are currently produced by the cracking of petroleum products. On the other hand, rubber trees produce hydrocarbons directly rather than carbohydrates. There are many latex-bearing plants (some 30,000 species) whose latex does not produce rubber but are nevertheless potential sources of hydrocarbons. Although it has a similar chemical composition as petroleum, the Hevea hydrocarbon polymer has a somewhat different atomic arrangement and molecular weight distribution from the hydrocarbons in petroleum.³ If, for example, the molecular weight distribution of the Hevea hydrocarbon could be reduced to less than 10,000 instead of 100,000 and 1,000,000 and the products harvested, we might have a "fuel" tree.

Furthermore, if one accepts these plants as a source of hydrocarbon rather than as a source of elastomer (rubber), the fact that the yield of plantation Hevea was raised in the last thirty years from 400 lbs/acre/year to 24,000 lbs/acre/year is an indication of the potential inherent in this species through breeding.

Towards this end we have carried out a detailed examination of the low molecular weight lipid components in the latices of Hevea brasiliensis and Euphorbia coerulescens. The aims of this examination being to find out: (a) what is in the latex; (b) what is the distribution and structure of the high molecular weight polymers in the latices; and (c) is the distribution of the high molecular weight components influenced by the types of low molecular weight lipids present in the latex?

This article presents our initial findings from the analyses of low molecular weight lipids in latices from Hevea brasiliensis and Euphorbia coerulescens. A report on the distribution of the high molecular weight components will be published elsewhere.⁴

Results and Discussion

A sample of Hevea brasiliensis (RRIM 701) latex was obtained from the Rubber Research Institute of Malaysia. The sample of latex from Euphorbia coerulescens was collected from the Botanical Gardens at the University of California, Los Angeles. (A token specimen of Euphorbia coerulescens can be examined in the herbarium at the University of California, Berkeley.)

The latices were dissolved in ether or tetrahydrofuran and subsequently fractionated into neutral, basic and acidic fractions using standard separation techniques. An additional semi-polar fraction was obtained from the Hevea latex by sonication of the high molecular weight precipitate (rubber) with a mixture of heptane, ethyl acetate, and methanol. The neutral and organic acid fractions were further fractionated by urea adduction. The fractions so obtained were purified by column chromatography and thin-layer chromatography prior to methylation and derivatization where necessary.

The quantitative distribution of the various fractions from each latex is given in Table I. Water and "high molecular weight" components, or non-THF soluble components, account for almost 95% of the material in each latex. The remaining material was composed of soluble lipids and insoluble proteins and carbohydrates.

Since the main idea of the project is to determine the potential usefulness of these "high molecular weight components" as a source of hydrocarbons, information on the distribution of lower molecular weight compounds and the way in which they are related to the distributions of these components will be of the utmost importance. Table II summarizes the major low molecular weight components identified in each fraction by gas chromatography (GC) and computerized-gas chromatography-mass spectrometry (C-GC-MS). The most significant points to emerge from this data are:

- 1) Absence of any significant quantities of isoprenoid hydrocarbons or fatty acids. The "high molecular weight" components are known to be poly-isoprenes, thus significant quantities of low molecular weight isoprenoidal components would have been expected to be present.

- 2) Major differences were observed between the sterol content of the latices. The major sterols in the Euphorbia latex were initially identified as being isomers of lanosterol and a homolog of an isomer of lanosterol.^{5,6,7} Further work, including co-injection with authentic standards on a gas chromatograph, has shown that these sterols are euphol, euphorbol, and tirucallol. This extends the distribution of these sterols within the Euphorbiacea from that described by Ponsinet and Ourisson⁸ who reported euphol, euphorbol and tirucallol to be normally restricted to African cactus-like Euphorbiacea. The major sterol of Hevea was identified as β -sitosterol,

and no evidence was found to indicate the presence of cycloartenol as reported by Ponsinet and Ourisson.⁸

3) The most significant finding, and one which has not been reported before, was the presence of 2-methylcyclobutanone, 2-methyl-2-hydroxycyclobutanone, 2-methylcyclobutanol, in the Hevea latex. These components were only isolated after sonication of the high molecular weight residue. This suggests that they might have been formed by cyclisation of isoprene components, present in or formed from the rubber during this process.

A survey of many Euphorbia latices is now underway to determine the variation in distributions of the high molecular weight components. The parallel survey of the low molecular weight lipids will show whether or not they play an important role in this variation. As mentioned above, the ultimate aim of this project is to obtain a plant where the molecular weight distribution of the latex is such that these components can be successfully utilized as an alternative source of hydrocarbons.

Experimental

The sample of latex from the Euphorbia coerulescens was collected from botanical gardens at UCLA by tapping the plant and collecting the latex directly into tetrahydrofuran. The sample of latex from Hevea brasiliensis was collected by Dr. Subramaniam of the Rubber Research Institute of Malaysia.

The latices were extracted and separated, using standard separation techniques. Derivatization, where necessary, was performed with BF_3/MeOH (14% w/v) for methylation of organic acids and HMDS/IMCS for silylation of free hydroxyl groups. GC analyses were performed on a Varian 2700 GC

equipped with a flame ionization detector and linear temperature programmer. The analyses were performed on a micropacked capillary column, packed with 3% Dexsil 300 on 80-100 mesh Gas-Chrom Q. Combined GC-MS analyses were carried out on a DuPont 492-1 mass spectrometer interfaced with a Varian Aerograph Model No. 204 equipped with linear temperature programmer. The column used for the GC-MS analyses was a 9 m x 0.7 mm i.d. glass capillary column packed with 1% OV-1 coated on 80-100 mesh Gas-Chrom Q. The mass spectral data were acquired and processed using a DuPont 21-094 data system.

The components were identified from GC retention times, co-injection of standards where possible, interpretation of mass spectra and comparison of mass spectra data with that of standard compounds.

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Table 1 - COMPARISON OF YIELDS FROM VARIOUS FRACTIONS OF THE HEVEA AND EUPHORBIA LATEX

Species	Water soluble components (%) ¹	Water (%) ¹	Organic-solvent soluble components			
			"High molecular weight" (%) ²	Low molecular weight (%) ²		
				Neutral	Acidic	Basic
<u>Hevea brasiliensis</u>	3.4	64.8	30.7	0.5	0.3	0.3
<u>Euphorbia coerulescens</u>	9.2	63.2	0.4	27.0	0.1	0.1

¹ % Total Latex

XBL 767-6036

² "Higher molecular weight" is material ppted. from THF by CH₃OH-H₂O

Table II - MAJOR COMPONENTS IN HEVEA LIPID EXTRACT

Class of compound	Components
Hydrocarbons	<u>C₁₈H₃₆</u> , C ₂₀ H ₄₀ , C ₂₀ H ₄₂ (branched)
Sterols	Stigmasterol, <u>β-Sitosterol</u> , Fucosterol (<u>E. coerulescens</u> sterols identified as euphol, euphorbol, and tirucallol)
Fatty Acids	n-C ₁₆ , n-C ₁₈ and n-C _{<u>18:2</u>}
Esters	<u>Methyl palmitate</u> , Methyl stearate
Fatty acids as glycerides	n-C _{<u>18:2</u>} and n-C ₂₀
Terpenes	2-Methylcyclobutanone, <u>2-Methylcyclobutanol</u> 2-Methyl-2-hydroxycyclobutanone, C ₅ H ₁₂ O, C ₁₀ H ₂₀ O
Pigments	Carotenoids

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