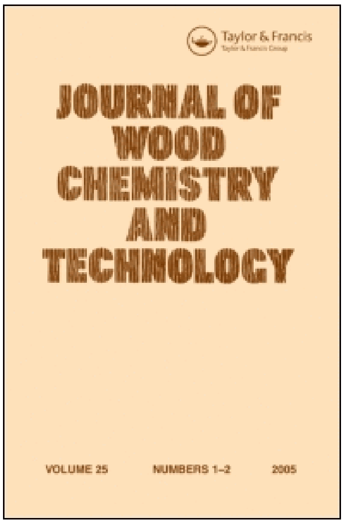

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N. Marwah[a]; A. Masohan[a]; D. Bangwal[a]; V. K. Bhatia[a]
[a] Indian Institute of Petroleum, Dehradun, India

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CHEMICAL COMPOSITION OF GUAYULE (PARTHENIUM ARGENTATUM GRAY) WAX

N. Marwah, A. Masohan, D. Bangwal and V.K. Bhatia*
Indian Institute of Petroleum
Dehradun 248 005 India

ABSTRACT

Commercialization of guayule holds promise not only as an additional source of natural rubber but also for the utilization of its byproducts viz. wax, resin and bagasse. Hence interest in guayule has been revived once again. The present paper discusses the isolation, characterization and possible applications of one of the byproducts - *guayule wax.*

INTRODUCTION

Guayule is one of the two major plant species known to contain substantial quantities of rubber[1,2] for commercial production, the other being hevea tree. Unlike hevea tree where rubber is contained in ducts and can be collected by bleeding without destroying the plant, the rubber in guayule is stored in closed cells and requires harvesting and processing of the whole

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* To whom correspondence may be addressed

shrub to a stage of total disintegration of the wood morphology. Hence, economy of the process depends on the whole plant utilization where all components of shrubs can be utilised for the highest possible return because guayule rubber makes up only a small fraction (15% by weight) of the entire plant. Other components of guayule are resin (10%), water solubles (10%), leaves (15%),cork (2%) and bagasse (48%).

Guayule wax, an important byproduct, is present as cuticle on the leaves and protects the plant from excess water loss during the day time. Due to lack of information available on the nature of guayule wax it was studied for its chemical nature and physical properties to explore its potential applications.

RESULTS AND DISCUSSION

Guayule wax was isolated from dry guayule leaves by extraction with cyclohexane followed by purification of the extract (4.5% dry weight basis) by solvent deoiling with methanol and recrystallization to obtain pear shaped crystals (2.5% dry leaves weight basis). Purification step was continued till the wax showed only one spot on TLC.

Physico-chemical properties of pure wax were determined by standard ASTM methods (Table-I). It was found to be a bright, sweet smelling product of high melting point (76^{o}C) and high molecular weight (680). It was readily soluble in benzene, chloroform and toluene at 30^{o}C. Lower temperatures resulted in incomplete dissolution. An iodine value of 2.5g/100g indicated the wax to be devoid of any unsaturation and the saponification value of 94.7 mg KOH/g suggested the wax to be an ester.

Comparison of physical properties (Table-I), e.g. melting point, congealing point, penetration number, refractive index, iodine value, ash content and colour of guayule wax with those of other popular commercial waxes like bees and carnauba wax

TABLE-I

Physico-Chemical Properties of Bees Wax, Carnauba Wax, Guayule Wax and Its Fractions

Physico-Chemical Property	Method	Bees Wax	Carrauba Wax	Guayule Wax	Fractions	
					Guayule Wax	Transesterified Guayule
					BPW	TEB
Melting point, °C	ASTM D-127	64.0	75.5	76.0	-	-
Congealing point, °C	ASTM D-938	62.0	68.9	73.5	-	-
Penetration no., dmm	ASTM D-1321					
25°C		20	9	5	-	-
45°C		41	13	7	-	-
Refractive index,	ASTM D-1747					
80°C		1.4400	1.4361	1.4365	-	-
Acid value, mg KOH/g	ASTM D-664	18.4	3.3	1.2	-	-
Saponification value, mg KOH/g	-	-	-	94.7	92.4	92.4
Iodine value, g/100g	IP.84	12.0	3.0	2.5	1.5	1.5
Ash, wt%	ASTM D-482	0.001	0.02	0.001	-	-
Color	ASTM D-1500	1.5	1.0	0.5	-	-

suggested that it resembles well with carnauba wax[3] making it potentially viable for commercial exploitation.

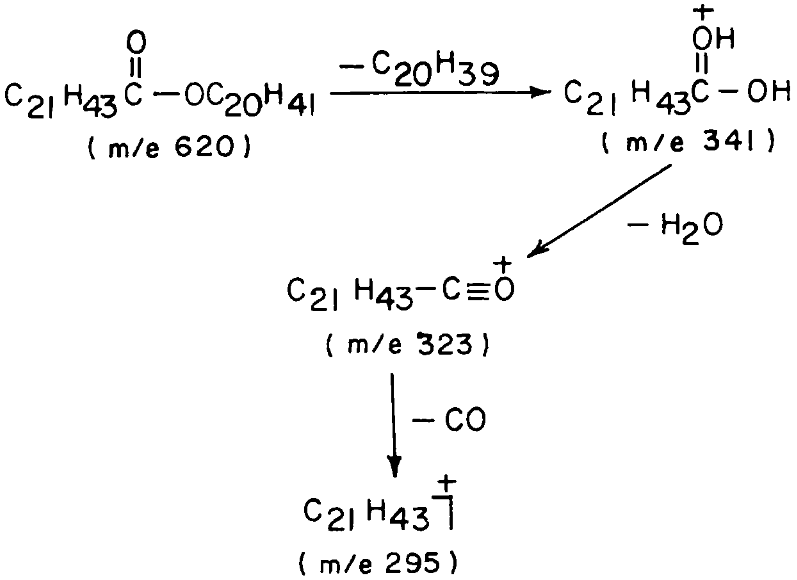
TLC behaviour of the wax in dichloromethane-heptane (1:9) at 50°C showed that it consisted of three types of components i.e. nonpolar (Rf, 0.9; probably paraffinic hydrocarbons), moderately polar (Rf, 0.5; probably esters) and highly polar (Rf,0.2). Esteric nature of the wax was also confirmed by the 1730, 1115 and 1050 cm^{-1} vibrations in its IR spectrum and protons at 4.0-4.3 ppm in its NMR spectrum.

Isolation and Identification of Constituents of Guayule Wax

Fractionation of wax at 50°C over silica gel columns resulted in eight fractions (APW, BPW, II CDE, III CDE, II CDEa, IICDEb, IV CDEa and IV CDEb). Out of these only two fractions, APW (3%) and BPW (92%), were in appreciable yield and were studied for characterization.

Infrared spectrum of APW did not show any functional groups except CH_3 and CH_2 of a saturated hydrocarbon (vibrations at 2960, 2862, 1462 and 1380 cm^{-1}). GC analysis of the fraction showed it to be a mixture of paraffins. These paraffins were $C_{19}H_{40}$ (2.6%), $C_{20}H_{42}$ (3.3%), $C_{22}H_{46}$ (0.7%), $C_{24}H_{50}$ (0.5%), $C_{25}H_{52}$ (12.7%), $C_{26}H_{54}$ (1.5%), $C_{28}H_{58}$ (2.0%), $C_{29}H_{60}$ (10.0%), $C_{30}H_{62}$ (3.0%), $C_{31}H_{64}$ (20.0%), $C_{32}H_{66}$ (2.0%), $C_{34}H_{70}$ (1.1%) and $C_{36}H_{74}$ (1.0 % by weight) in the mixture.

IR spectrum of fraction BPW showed peaks characteristic of CH_3 and CH_2 (2960, 2862, 1460 and 1380 cm^{-1}), $\overset{\overset{O}{\|}}{C}$-OR (1725, 1285 and 1045 cm^{-1}) and $(CH_2)_{n\geqslant 4}$ (720 cm^{-1})$_4$. Multiplets centered at 0.87 ppm (6 protons of methyl groups), between 1.10 and 1.57 ppm (74 protons of - CH_2 and -CH), centered at 2.10 ppm (2 protons of -CH_2-$\overset{\overset{O}{\|}}{C}$-O-) and centered at 4.0 ppm (2 protons



[Scheme-1]

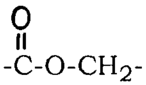
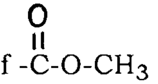
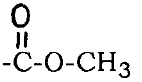
of $-\overset{O}{\overset{\|}{C}}-O-CH_2-$) were observed in the NMR spectrum of the ester[5]. Its mass spectrum showed base and molecular ion peaks at m/e 342 and 620 respectively. With two oxygen atoms of the ester group and 620 as the molecular weight, the molecular formula of BPW worked out to be $C_{42}H_{84}O_2$ (having one unsaturation, that of the carbonyl group). The base peak at m/e 341 is due to the characteristic behaviour of long chain esters[6-8] of the type $R'O\overset{O}{\overset{\|}{C}}R$ arising by the alkyl-oxygen cleavage and rearrangement of two hydrogen atoms [Scheme-1]. The other two major fragment ions at m/e 323 and 295 were explained by the loss of a water molecule from the m/e 341 fragment ion and a subsequent loss of acyl group respectively [Scheme-1].

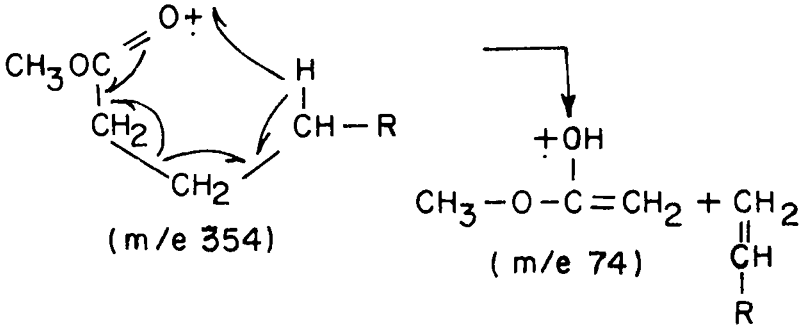
The m/e 295 ion peak showed a loss of 15 mass units followed by consecutive loss of 14 mass units suggesting it to be a straight chain alkyl group of formula $C_{21}H_{43}$. The structure of the fraction BPW which best satisfied all the spectral data was $C_{21}H_{43}COOC_{20}H_{41}$ (eicosanyl docosanoate).

Confirmation of Structures of Guayule Wax Constituents

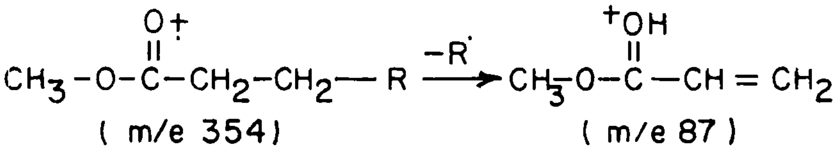
In order to confirm the structure of constituents of guayule wax identified above, it was subjected to saponification which could not yield appreciable amount of product even under drastic conditions as saponification was always incomplete. Therefore, transesterification (alcoholysis in presence of an acid to yield the respective methyl ester and alcohol by cleavage of the ester group) of the wax was tried. This resulted in six components in the product mixture i.e., TEA, TEB, TEC I, TEC II, TEC III and TEC IV. The first four of these were obtained in good yield and studied for detailed structural analysis.

Fractions TEA and TEB resembled fractions APW and BPW of the guayule wax in their physico-chemical properties as well as GC, elemental and spectral analysis suggesting the former to be mixture of paraffinic hydrocarbons in the same carbon number range as APW and the latter to be eicosanyl docosanoate.

Fraction TEC I showed strong bands characteristic of CH_3 and CH_2 (2951, 2872, 1480 and 1380 cm^{-1}) and  $-\overset{\overset{O}{\|}}{C}-O-CH_2-$ (1730, 1115 and 1050 cm^{-1}) in its IR spectrum and multiplets for 3 protons of alkyl $-CH_3$ (0.87 ppm), 38 methylene protons (1.20-1.40 ppm), 2 methylene protons of  $-\overset{\overset{O}{\|}}{C}-O-CH_3$ (2.10 ppm) and 3 methyl protons of  $-\overset{\overset{O}{\|}}{C}-O-CH_3$ (3.70 ppm) in its NMR spectrum. Thus the fraction was a methyl ester of a long chain acid. The molecular formula assigned to the fraction, based upon its elemental analysis (Table-II) and molecular ion peak at m/e 354 in the mass spectrum, was $C_{23}H_{46}O_2$ with one unsaturation of the carbonyl group. This was confirmed by the base peak at m/e 74 which is characteristic of methyl esters of long chain fatty acids and is formed by 2-3 cleavage with rearrangement of 1 H atom :



Formation of the other major fragment ion at m/e 87 was explained as below :



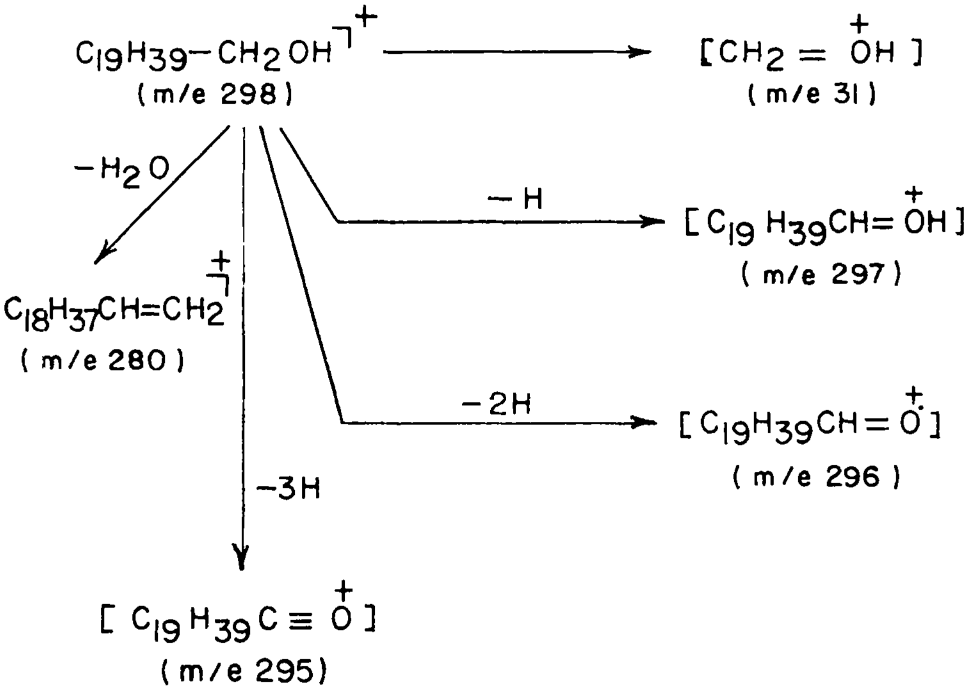
$$\underset{(m/e\ 354)}{CH_3-O-\overset{\overset{O^{+\cdot}}{\|}}{C}-CH_2-CH_2-R} \xrightarrow{-R^{\cdot}} \underset{(m/e\ 87)}{CH_3-O-\overset{\overset{^{+}OH}{\|}}{C}-CH=CH_2}$$

The parent ion peak of m/e 354 also showed a loss of 15 mass units (of $-CH_3$) to give a fragment ion at m/e 339 which subsequently showed consecutive loss of 14 mass units (of $-CH_2-$) to yield fragment ion at m/e 59 suggesting 20 $-CH_2-$ groups of a

TABLE-II

Elemental Analysis and Spectral Data of Guayule Wax Fractions

	IR	NMR		Mass	Elemental Analysis
	Frequences (cm^{-1})	Position	Protons	(m/e)	(Wt%)
Guayule wax	2980,1730,1480	0.8-1.0			
	1200,1115,1050	1.1-1.8			
		2.1-2.6			
		4.0-4.3			
APW	2960-2862	0.87			
	1462,1380	0.98			
		1.07-2.05			
BPW	2960,2862,1730,	0.87	6	620 (Parent ion),	C, 81.3; H, 13.5;
	1480,1380,1285,	1.10		341 (Base), 323,	0 (By difference), 5.2
	1040,720	1.57	74	295	[Calculated for
		2.10	2		$C_{42}H_{84}O_2$; C, 81.29,
		4.00	2		H, 13.55; O, 5.16]
TEA	2960,2862,1462, 1380	0.88, 0.98			
		1.07-2.05			
TEB	2960,2862,1730,	0.87	6	620 (Parent ion),	
	1480,1380,1280,	1.10		341 (Base), 323,	
	1045,720	1.57	74	295	
		2.10	2		
		4.00	2		
TEC-I	2951,2872,1730	0.87	3	354 (Parent ion),	C, 78.0; H, 13.0;
	1480,1380,1280,	1.20-1.40	38	74 (Base),	0 (By difference), 9.0
	1115,1050	2.10	2		[Calculated for
		3.70	3		$C_{23}H_{46}O_2$; C, 77.94;
					H, 12.99; O, 9.04]
TEC-II	3640,2959,2930	0.88	3	298 (Parent ion),	C, 80.5; H, 14.1;
	1480,1380,1050	1.20-1.40	36	31 (Base), 297,	0 (By difference), 5.3
		4.70	3	296,295	[Calculated for
					$C_{20}H_{42}O$; C, 80.54;
					H, 14.03; O, 5.43]



SCHEME-2

long chain alkyl group attached to $-O\overset{O}{\overset{\|}{C}}CH_3$. Hence the final structure assigned to TEC I was $C_{21}H_{43}\overset{O}{\overset{\|}{C}}OCH_3$.

The IR spectrum of fraction TEC II showed absorption bands for - OH group at 3640 cm^{-1} besides the 2957, 2930, 1480 and 1380 cm^{-1} vibrations for the alkyl $-CH_3$ and $-CH_2-$ groups. Its NMR spectrum showed 3 protons of $-CH_3$ (0.88 ppm), 36 protons of $-CH_2-$ (1.20-1.40 ppm) and the hydroxyl proton along with two protons of $-CH_2-O-\overset{O}{\overset{\|}{C}}$ at 4.7 ppm. Molecular weight of the compound was 298. With one oxygen of the hydroxyl group, the molecular formula of the compound was $C_{20}H_{42}O$. The parent ion peak was observed at m/e 298 in the mass spectrum with the base peak at m/e 31, characteristic for CH_2OH+ fragment of the primary alcohols. Peaks at m/e 297, 296 and 295 were due to the loss of one, two or three hydrogen atoms from the molecular ion, a behaviour that is characteristic of primary alcohols [Scheme-2].

Length of the alkyl chain of the alcohol was obtained by the fragmentation pattern of the alkyl group (loss of one $-CH_3$ and 18 $-CH_2-$) from the parent ion to yield the m/e 31 peak. Thus the alcohol was eicosan -1-ol $[CH_3(CH_2)_{18}CH_2OH]$.

Thus the transesterified wax was a mixture of : i) hydrocarbons present in the starting guayule wax which remained unaffected in the transesterification reaction, ii) unconverted ester (eicosanyl docosanoate) iii) converted products of methyl docosanoate and eicosan -1- ol and iv) the minor constituents which were not identified either in the guayule wax or in its transesterified product mixture.

EXPERIMENTAL

Guayule shrubs with leaves were received from the National Botanical Research Institute, Lucknow, India. The leaves (15%, fresh shrub weight basis) were removed manually from the branches and air dried. These were finally dried at 40^{0}C to constant weight in an oven. Solvents and reagents used were BDH [British Drug House, Laboratory reagent (LR) grade]. Molecular weights of pure components were determined by Vapour Pressure Osmometry (VPO) and elemental analysis by Heraus CHNO (Carbon, hydrogen, nitrogen, oxygen) rapid analyser. Spectral analysis was done on Perkin Elmer model 399B infrared spectrophotometer, Jeol Fx-100 Nuclear Magentic Resonance (NMR) spectrometer (in deuterated chloroform) and

Hitachi RMU-CE model Mass spectrometer (at 1,7 and 70 eV). Gas chromatographic analysis was carried out on Perkin Elmer model 3920B Gas chromatograph using 100% methyl silicone column (2mx2mm i.d.) with temperature programmed from 150 to 300^{0}C at a rate of 3^{0}C/minute.

Isolation and Purification of Wax

Guayule leaves (5 kg) were subjected to extraction with cyclohexane [4x2.5l). Solvent was removed from the combined extracts by distillation to yield a residue (4.5% dry leaves weight basis). The residue was dissolved in 100 ml of chloroform and poured over 3 l of warm methanol with constant stirring when the wax precipitated out and the impurities remained dissolved in methanol. The precipitated wax was filtered and the process repeated 2-3 times. Finally the wax was washed with warm methanol to obtain pure wax (2.5% dry leaves weight basis). Purification step was monitored by TLC using silica gel as adsorbent and methanol as the developing solvent. While the extract showed two spots (Rf 0.8 and 0.1), the wax and methanol soluble parts showed one spot each (Rf 0.1 and 0.8 respectively). The purified wax was finally recrystallized from chloroform as fine pear shaped crystals. The wax was soluble in chloroform and benzene and had pleasant odour. It showed three spots with Rf values 0.9, 0.5 and 0.2 on TLC plate at 50^{0}C with silica gel as adsorbent and dichloromethane : heptane (1:9) as the developing solvent. Physical properties of the wax are given in Table-I and its infrared and NMR spectral data are presented in Table-II.

Fractionation and Characterization of Wax

Pure guayule wax was subjected to column chromatography over silica gel (A/S = 50) at 50^{0}C. Fraction APW (3% by weight) obtained by elution with heptane and BPW (92% by weight) by elution with heptane : dichloromethane (17:3) were the major fractions and were studied further. GC analysis of fraction APW suggested it to be a mixture of paraffins of carbon number C_{19}-C_{36}. Tables I and II show the physico-chemical properties, elemental analysis and IR, NMR and mass spectral data of the two fractions.

Saponification and Transesterification of Guayule Wax

2 G of wax was saponified by adding 5% alcoholic potash solution and dissolving in 60 ml of benzene. The mixture was refluxed for 3 hrs on a water bath. Similar experiments were repeated using higher concentration of alkali (15 and 30%) and longer refluxing times (6 and 12 hrs). The resulting reaction products, after work-up, were found to contain appreciable amount of unconverted wax in all the cases.

For transesterification 2 g of guayule wax was refluxed with 20 ml methanolic HCl (hydrochloric acid) and 5 ml benzene for 8 hrs. The reaction product mixture, thus obtained, was made solvent and acid free.

Fractionation of Transesterified Wax

The transesterified wax obtained above was fractionated by column chromatography at 50°C over silica gel adsorption columns (A/S = 50). Successive elution with heptane, heptane: dichloromethane (3:17) and (1:1) afforded fractions TEA, TEB, and TEC respectively. Fraction TEC was further fractionated by eluting over silica gel column (A/S = 50 and temperature, 50°C) with benzene : heptane (1:1) and isopropanol : heptane (1:19) to yield fraction TEC I and TEC II respectively. The physical properties, elemental analysis and spectra of all the above fractions are given in Tables-I and II. GC analysis of fraction TEA revealed it to be a mixture of paraffinic hydrocarbons in the C_{19}-C_{36} range. The chromatogram resembled that of APW.

CONCLUSION

Guayule wax has been isolated and fractionated for detailed characterization of its components. The wax has been found to consist of a hydrocarbon part (3%) which is a mixture of paraffinic hydrocarbons in the C_{19}-C_{36} carbon number range, an esteric part (92%) which is mainly the eicosanyl docosanoate and a highly polar part. Transesterification of the ester yielded methyl docosanoate and eicosan - 1-ol. The guayule wax has good potential for economic exploitation because of its resemblance with carnauba wax in physico-chemical properties.

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