Quantitative and Qualitative Variations in Resin Content and Guayulins (A and B) among Different Guayule Cultivars

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Guayule has substantial quantities of resin which is often discarded after rubber extraction. Resin from 13 cultivars of guayule from India was analyzed for its qualitative and quantitative variations. Stem had 6.15% resin followed by roots (5.91%), branches (5%), leaves (3%), and flowers (3.06%). Resin percent varied significantly among the cultivars. It varied from 10.7% in USDA-10 to 5.5% in UCR-1; however, resin synthesis and biomass of the shrub had poor correlation. Guayulins (sesquiterpene cinnamic acid esters) were analyzed by HPLC. Composition of guayulins varied among cultivars. Guayulin A was in the range 0.27−3.7 g/plant, the lowest being in USS2X and the highest in USDA-17. Regression analysis between resin yield and guayulin A as well as guayulin B per plant showed poor correlation. Guayulin A dominated over guayulin B in all plant parts analyzed. Variation in resin percentage and guayulin composition in different cultivars may be due to differences in the origin of the cultivars and not necessarily due to temperature or other environmental factors. Resin synthesis appears to be independent from rubber synthesis and is not influenced by temperature minima, which is obligatory for *cis*-polyisoprene biosynthesis.

Keywords: *Guayule; resin; guayulins; chromatography*

INTRODUCTION

As a renewable source of natural rubber, the Chihuahuan desert shrub guayule (*Parthenium argentatum* Gray) of Asteraceae is undergoing careful economic assessment throughout the world. Apart from rubber, guayule has a substantial quantity of resin which is often rich in sesquiterpenoids and triterpenes. The resin is often discarded as a byproduct in the processing of rubber.

Guayule resin has been evaluated by several investigators with regard to its specific applications. Bultman *et al.* (1982, 1991) found that resin is a potentially important wood protector for marine and terrestrial environments. Belmares *et al.* (1980) incorporated guayule resin into coating formulations for structure-property relationship studies. Pesticidal properties of guayule resin have been reported by Bultman *et al.* (1991). According to them, the resin protects wood from termite attack, particularly the more destructive *Coptotermes* species. They also reported its biocidal properties against teredinids and limnorians, brown-rot, soft-rot, and white-rot fungi.

A variety of secondary metabolites have been isolated from the resin and studied by various workers. Several monoterpenes, including α-pinene, have been reported from guayule resin (Scora and Kumamoto, 1979). In recent years, several nonrubber isoprenoid compounds have been reported from guayule. The major among these are terpenes and sesquiterpenes (Haagen-Smith and Siu, 1944; Schloman *et al.*, 1983); sesquiterpene esters, namely guayulin, A and B (Rodriguez *et al.*, 1981); diterpene ketoalcohols (Dorado, 1962); phytosterols (Buchanan *et al.*, 1978); and tetracyclic triterpene derivatives of cycloartenol, the ketoalcohols (Romo de Vivar *et al.*, 1990). α-Linoleic acid was determined to be the principal fatty acid component of the various esters present (Banigan and Meeks, 1953; Banigan *et al.*, 1982). Considered separately, each chemical class of resin components has potential commercial value.

The two sesquiterpene phenolic acid esters, germacrene cinammic acid ester (guayulin A) and germacrene *p*-methoxybenzoic acid ester (guayulin B), were extensively studied (Schloman *et al.*, 1983). The report on these being potent contact allergens by Rodriguez *et al.* (1981) sparked further interest in these metabolites. Proksch *et al.* (1981) and Behl *et al.* (1983) reported an HPLC separation system for quick isolation and quantification of these metabolites. They also reported their inheritance patterns by studying hybrids and the parent species from the United States.

There are several cultivars being grown in various agroclimatic zones in India. Sidhu *et al.* (1993) have reported qualitative and quantitative variations in rubber of cultivars available in India. However, there has been no study on similar aspects with respect to resin. We have studied resin of 13 cultivars of guayule from India for its quantity and qualitative variations with respect to guayulins. The same is reported in this paper.

MATERIALS AND METHODS

Thirteen guayule cultivars, namely ARIZ-101, CAL-2, GH-9, USDA-5, USDA-10, USDA-17, C-244, ALI-10, G-97, N575, USS2X, GILA, and UCR-1, are being maintained at the experimental station of the Biomass Research Center of this Institute. USDA-5, USDA-10, USDA-17, UCR-1, UCR-7, and N575 were originally obtained from the University of California, Riverside, while C-254, C-244, ALI-10, and USS2X were obtained from University of California, Davis, Schafter station. ALI-10 and USS2X are not the standard cultivars; rather they were collections and were obtained from Dr. Ali Estilai, University of California, Riverside. USS2X was a relatively pure diploid cultivar. Source of G-series is not known. Various plant parts were harvested from 4-year-old plants during December 1991 through January 1992. Samples were also

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0021-8561/95/1443-2012$09.00/0

Table 1. Biomass, Resin Yield, and Guayulins (A and B) among Different Guayule Cultivars

guayule cultivars	biomass (g/plant)	resin (%)	resin (g/plant)	guayulins (g/plant) guayulin A	guayulins (g/plant) guayulin B
USDA-10	706.5 ± 9.40[a]	10.68 ± 0.26	75.45 ± 1.85	2.97 ± 0.007	0.53 ± 0.007
G-97	843.5 ± 11.17	9.97 ± 0.18	84.09 ± 1.27	0.62 ± 0.004	0.13 ± 0.011
USDA-17	746.6 ± 9.33	8.94 ± 0.13	66.74 ± 0.81	3.79 ± 0.101	0.75 ± 0.017
C-244	666.5 ± 12.15	8.92 ± 0.56	59.45 ± 0.13	1.01 ± 0.016	1.00 ± 0.024
USDA-5	723.0 ± 27.21	8.07 ± 0.13	58.34 ± 0.45	2.90 ± 0.153	1.17 ± 0.016
GH-9	996.5 ± 50.45	7.30 ± 0.18	72.74 ± 0.66	1.64 ± 0.016	0.99 ± 0.017
ALI-10	889.6 ± 23.44	7.06 ± 0.08	62.80 ± 0.66	0.85 ± 0.015	0.28 ± 0.008
CAL-2	992.4 ± 44.36	7.00 ± 0.15	69.46 ± 0.28	0.51 ± 0.009	0.52 ± 0.005
ARIZ-101	926.5 ± 40.07	6.80 ± 0.31	63.00 ± 0.24	2.06 ± 0.046	1.01 ± 0.011
N575	826.3 ± 32.60	6.40 ± 0.16	52.88 ± 0.20	3.12 ± 0.032	1.38 ± 0.014
USS2X	514.5 ± 20.68	6.22 ± 0.16	32.00 ± 0.29	0.27 ± 0.011	0.37 ± 0.014
GILA	912.6 ± 28.83	6.15 ± 0.15	56.12 ± 0.45	2.08 ± 0.045	1.78 ± 0.019
UCR-1	817.6 ± 16.96	5.50 ± 0.21	44.96 ± 0.28	2.15 ± 0.070	0.89 ± 0.009

[a] ± = standard error.

taken from 12-, 24-, 36-, and 45-month-old plants to study variations in metabolites at various ages. Stem, root, branches, and flowers were separated and dried in an oven at 35 °C.

Samples were then ground to fine powder in a Wiley type mill (Yorko Instruments, New Delhi, India) with a 2-mm mesh screen and again dried in an oven at 35 °C until a constant dry weight was achieved. There were four replications for each experiment.

Samples were extracted in acetone (Qualigens Fine Chemicals, Bombay, India) using a Kinematica PT-MR 6000 Polytron homogenizer (Littau, Switzerland) equipped with PT-DA 3007/2 aggregate. The extracts were filtered and left overnight at low temperature. The samples were filtered through a 0.5-mm Teflon filter to remove waxes.

Qualitative Analysis. Analyses were performed on a Waters liquid chromatograph equipped with a Waters automated gradient controller, a Waters 501 solvent delivery system, a Rheodyne 7125 sample injector fitted with a 20-μL loop, a Waters 484 variable absorbance detector, and a Waters 740 data module. A reversed-phase C_{18} column (3.9 x 300 mm) equipped with a Waters precolumn was used. A KT-25S degassing device (Shodex degasser, Tokyo, Japan) was used to degas the solvents. The mobile phase consisted of an isocratic mixture of CH_3OH-H_2O (89:11) with a flow rate of 1.0 mL min^{-1}.

Guayulin A has a λ_{max} = 275 nm, and the λ_{max} of guayulin B is 250 nm; however, 254 nm gave a reasonable representation, and the variations in the extinction coefficients were within reasonable limits. The results are presented as percent weight basis, and the same were quantified using external standards of guayulins A and B.

Guayulins A and B were isolated from the guayule resin by column chromatography. The column was eluted with chloroform, followed by increasing amounts of acetone. The compounds were confirmed against standards obtained from Prof. Eloy Rodriguez, Phytochemistry and Toxicology Laboratory, University of California, Irvine, CA.

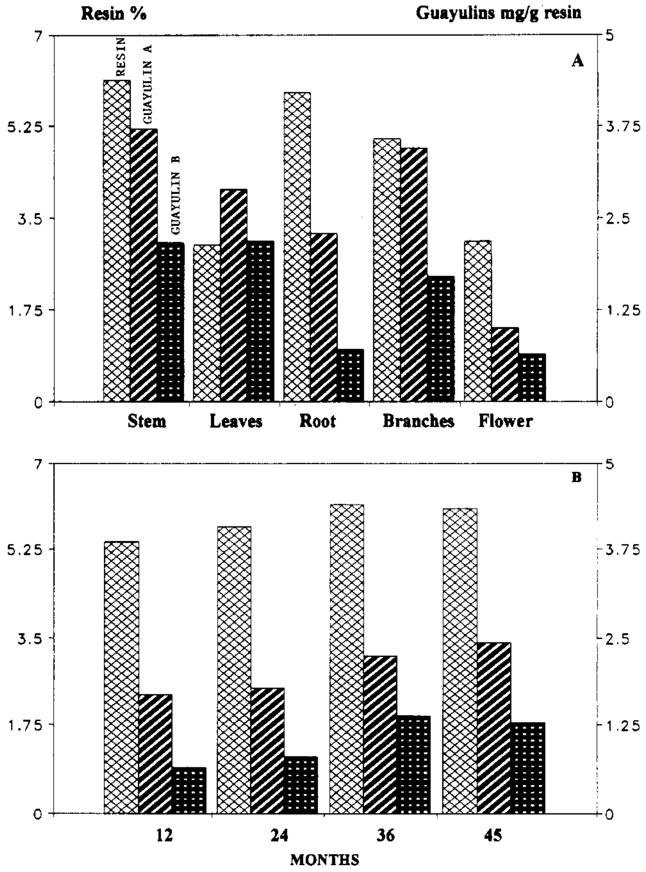

Figure 1. Resin and guayulin A and B content in GILA cultivar of guayule: (A) analysis of various plant parts; (B) analysis of plants at various growth stages.

RESULTS AND DISCUSSION

Resin Content. Resin percentage varied significantly among the 13 cultivars investigated. Total extracted resin calculated as percentage of shrub dry weight as well as total resin per plant is shown in Table 1. Percent resin ranged from 5.5 in cultivar UCR-1 to 10.7% in USDA-10. USDA-10 with 10.7% resin and with 75 g of resin per plant stands out for it can yield up to 7.5 quintals of resin/h. When calculated on a per plant basis, USS2X had the minimum value of 32 g, while G-97 with 84 g of resin/plant was on the other extreme among the 13 cultivars. USS2X, a diploid cultivar, is slow growing and had an average biomass of 514 g/plant, which was nearly half of that in other promising cultivars. This cultivar also had higher (40%) mortality, which further reduced resin yield when calculated on a per hectare basis. However, there was no direct correlation between resin synthesis and biomass of the shrub. Regression output with dry weight per plant as an independent variable and resin percent as a dependent variable had R^2 = 0.058.

Resin content in stem, root, branches, leaves, and flowers was analyzed to evaluate proportional accumulation of resin in various plant parts. Analysis of GILA cultivar (Figure 1A) showed that on a percent dry weight basis, stem had 6.15% resin followed by roots (5.91%), branches (5%), leaves (3%), and flowers (3.06%).

Qualitative Determination of Guayulins. Guayulins (sesquiterpene cinnamic acid esters) are the most significant metabolites. These can be regarded as chemical markers for breeding programs as none of the other *Parthenium* species has these metabolites. Chromatographic separation was improved from the earlier reported studies. An isocratic mixture of methanol–water gave better resolution than acetonitrile–water. The flow rate could be maintained at 1 mL min^{-1} instead of 2.5 mL min^{-1} on a Lichrosorb RP-18 column as reported by Proksch *et al.* (1981).

Relative proportions of guayulins from 13 cultivars are shown in Table 1, and the same from various plant parts and at four stages of growth (up to 45 months) in a sample cultivar are shown in parts A and B of Figure 1, respectively. Guayulin A composition varied among cultivars. It was in the range 0.27–3.7 g/plant, the lowest being in USS2X and the highest in USDA-17. The range for guayulin B was between 0.13 and 1.77 g/plant, the lowest in G-97 and the highest in GILA. Guayulin A dominated over guayulin B in most of the cultivars except in USS2X, where guayulin B was 0.37 g/plant as compared to 0.27 g/plant for guayulin A. In certain cultivars, such as C-244 and CAL-2, guayulin A was almost equal in proportion to guayulin B.

Regression analysis between resin yield and guayulin A as well as guayulin B per plant with resin as an independent variable showed poor correlation between these two parameters. The R^2 value was 0.004 when resin was compared against guayulin A and 0.072 when compared against guayulin B.

Stem, root, leaves, tertiary branches, and flowers of GILA cultivar were analyzed for a comparative evaluation of guayulins accumulated in resin from these tissues. Guayulins were the major metabolites in the resin that oozes from the stem at any point of injury. Guayulin A dominated over guayulin B in all plant parts analyzed (Figure 1A). Guayulin A exhibited more than a 3-fold increase over guayulin B in root tissues with 2.3 mg g^{-1} of resin of guayulin A and 0.7 mg g^{-1} of guayulin B. This increase was only 1.5 times in stem, while the differences among these two metabolites in leaf tissues were minor, with 2.9 mg g^{-1} of guayulin A against 2.2 mg g^{-1} of guayulin B. Rodriguez *et al.* (1981) reported that guayulin A is present in stems and leaves at 0.05 and 0.03% dry weight, respectively. Schloman *et al.* (1983) reported that guayulin A composition varied from 8 wt % in line 12229 to 10 wt % in N575 and 11635. The same for guayulin B was 1–3 wt % in N575 and 11635, respectively. Guayulin A has been reported to be one of the major components of the processed resin byproduct obtained from the Saltillo guayule pilot plant in Saltillo, Mexico (Rodriguez *et al.*, 1981).

Resin accumulation increased with the age of the guayule plant. It was 5.4 and 5.7% in the first 2 years. However, it increased to 7.3 and 7% dry weight of shrub in the third and fourth years, respectively. Guayulin A increased with age from 1.7 mg g^{-1} resin in the first year to 1.8, 2.25, and 2.4 mg g^{-1} of resin in the following years. There was a similar response in guayulin B (Figure 1B).

Most of the recommended cultivars had high biomass but not necessarily high resin synthesis. GILA and ARIZ-101, the most popular cultivars in the United States, had high biomass but relatively poor resin. Similarly, test cultivar UCR-1, which had the least resin percentage of the 13 cultivars, had above average biomass. Regression analysis also revealed that there was no correlation between biomass and resin percentage. Earlier, Foster *et al.* (1986) reported that resin (and rubber) yields of the USDA and Anderson lines were extremely variable. Average resin ranged from 5.0 to 11.2% in the Anderson 78 and USDA 11646 lines, respectively. They divided their collections into different morphological groups based on leaf and inflorescence phenology and canopy characters; however, they did not report any positive correlation of any of the phenological characters with resin content. Sidhu *et al.* (1993) reported that there was poor correlation between rubber and resin synthesis in these cultivars.

Enzymatic potential for *cis*-polyisoprene (rubber) biosynthesis was shown to be effected by the temperature minima and the duration of low temperature to which plants were exposed; however, the same was not true for *trans*-terpene resin (Appleton and Staden, 1989). The initial steps leading to resin biosynthesis are not limited by temperature variables. Benedict *et al.* (1986) also reported that low temperatures do not stimulate the synthesis of *trans*-terpene resin. Some of the earlier reports regarding temperature requirement and minimum or maximum threshold for resin accumulation potential are conflicting and contradictory. Temperature in local conditions where this study was conducted in India remains above 30 °C for most of the year except for a few winter months when the average temperature is 18.7 °C. It rarely drops below 6 °C. Plants remain exposed to high temperature, often exceeding 40 °C, in summer months (May–June).

USS2X and UCR-1, which are the diploid cultivars (Ratti, 1993), tend to have lower proportions of resin. On the other hand, cultivars that are progenies of hybrids of guayule with other tree-form species of *Parthenium* such as *P. tomentosum, P. fruticosum,* and *P. schottii* had higher resin percentage. They appear to have inherited higher *trans*-terpene biosynthesis potential from the nonrubber and relatively high resin producing species. Differences in origin of a cultivar and the hybridization it may have undergone, not necessarily be reflected in its ploidy, however, could explain the variations in resin synthesis potential. Study of the available germplasm is crucial to assess the quality and quantity of resin to characterize the cultivars and their products.

Resin concentration has been shown to vary with age. Guayulin composition registered corresponding increases. Since mature plants synthesize more rubber than young plants, one would expect that when rubber accumulation accelerates, resin concentration or metabolism of terpenes and sesquiterpenoids might decrease. However, we found that resin as well as concentration of guayulins increased steadily with age. It was not affected by increase in rubber synthesis, and thus, the two are not antagonistic. Earlier, Arreguin *et al.* (1951) reported that the biochemical pathways leading to rubber formation are of minor importance relative to other isoprenoid synthesis.

Resin from guayule has several applications; it may become a valuable resource if high resin producing cultivars with promising biomass are selected for warm tropical habitats. Guayulin composition and localization appear to be very specific in plant parts and cultivars. Schloman *et al.* (1986) reported that the level of terpenoids does not alter with seasons. Resin synthesis does not interfere with rubber accumulation as the potential for rubber and resin accumulation differ in temperature requirements. Thus, guayulins can be used as markers in resin evaluation and breeding studies of guayule.

ACKNOWLEDGMENT

We are grateful to Dr. P. V. Sane, Director of the Institute, for advice and facilities. We thank Prof. Eloy

Rodriguez, University of California, Irvine, CA, for providing standards of guayulins.

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Received for review December 5, 1994. Accepted May 12, 1995.[⊗] We are grateful to the Department of Science and Technology and Department of Non-Conventional Energy Sources for funding the research.

JF940685K

[⊗] Abstract published in *Advance ACS Abstracts,* June 15, 1995.