Inheritance of Sesquiterpenoid Phenolic Acid Esters (Guayulins) in F_1 Hybrids of *Parthenium* (Asteraceae)

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High performance liquid chromatography was used to quantify the sesquiterpene phenolic esters, Guayulin A and -B in *Parthenium argentatum* (guayule) and F_1 hybrids of guayule with *Parthenium schottii, P. fruticosum* var. *trilobatum and P. tomentosum* var. *stramonium*. The distribution of these phenolic acid esters in all parts of the plants are reported and their inheritance in hybrids is discussed.

Introduction

Guayule (*Parthenium argentatum*(Gray)-Asteraceae), a shrub native to the Chihuahuan desert of Northern Mexico and Southwestern US, is presently being considered as an alternative source of natural rubber. Following the initiative of Mexico and the United States, many other countries in the world have started research and cultivation of this shrub and its relatives. Besides guayule only *P. rollinsianum* (Rzedowski) is known to produce small amounts of rubber [1]. In order to improve the rubber and resin production, guayule has been hybridized with numerous arborescent species of *Parthenium* at the Guayule Breeding Program, University of California, Riverside. The sesquiterpene lactone and resin chemistry of many of the species of *Parthenium* and their chemotaxonomical relationships have been investigated by Rodriguez [2], but little is known on the inheritance of the terpenoid secondary metabolites in the hybrids of guayule. Apart from the quantitative studies on rubber content of the F_1 hybrids we have also been investigating the inheritance of the resin components. In this communication, we report on the H.P.L.C. analysis and inheritance pattern of guayulins A and B in F_1 hybrids of guayule when crossed with the species *P. schottii* (Greenman) of Yucatan, Mexico; *P. fruticosum* var. *trilobatum* (Less.) of Tamaulipas, Mexico; and *P. tomentosum* var. *stramonium* (Greene) of Sonora and Chihuahua, Mexico. These sesquiterpenoic esters were first reported in guayule by Romo *et al.* [3] and most recently have been established to cause allergic contact dermatitis in experimental animals [4]. Dermatological experiments using Freund's complete adjuvant technique (FCAT) showed guayulin A to be as potent elicitor as pentadecylcatechol from poison ivy (*Toxicodendron radicans*). Recently we reported a high pressure liquid chromatographic method to detect guayulins in crude plant extracts [5]. In order to use this technique for preparative scale and quantification we have further improved the extraction procedure and the H.P.L.C. separation of the guayulins.

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Materials and Methods

Three year old plants of guayule, F_1 hybrids of *P. tomentosum* var. *stramonium* and *P. fruticosum* var. *trilobatum*, and 18 month old *P. schottii* hybrids were obtained from Los Angeles State and County Arboretum, Arcadia, California, then cultivated at the University of California, Riverside where the herbarium specimens were deposited. Roots, stems, leaves and inflorescence of each plant were separated and air dried. The resulting materials were ground in a Wiley Mill. A known quantity of dry material was extracted three times with benzene (50 ml/g) using Polytron, Brinkman Instruments, N.Y., for five min at 10000 rpm. The extract was centrifuged at $5000 \times g$ for fifteen min; the supernatant was completely evaporated under mild temperature (below 60 °C). Residue was then dissolved in a known volume of methanol using ultrasonic bath. Before injection, samples were filtered through millipores to remove waxes. H.P.L.C. analyses were performed on Beckman gradient liquid chromatograph series 334 equipped with 421 controller, 110 pumps, 210 sample injection valve fitted with a 25 µl loop. Detection was achieved using a Hitachi variable wavelength detector model 100-10. The chromatographic column consisted of Altex Ultrasphere ODS type (4.6 × 150 mm, 5 µ $\bar{d}$p). Elution was achieved using an isochratic mixture of methanol and water (93:7) as mobile phase at a flow rate of 1 ml/min. Quantification was done with Shimatzu integrator model C-E1B using external standards. Cross sections of guayule stem were fixed and processed through customary methods for SEM preparation and photographed on Zeiss Scanning Electron Microscope.

Results and Discussion

Roots, stems, leaves and inflorescence of guayule and of the F_1 hybrids of guayule with *P. tomentosum*, *P. fruticosum* and *P. schottii* were quantitatively analysed for guayulin A and -B as reported in Tables I and II. Chromatograms were recorded at 254 nm (λ_{max} of guayulin A is 275 nm; λ_{max} of guayulin B is 250 nm) so that they could be repeated even with a fixed wavelength detector. At this wavelength, the extinction coefficient of guayulin B happens to be about three times that of guayulin A. The use of a shorter column (15 cm instead of 25 cm) provided a rapid and better resolution as shown in Fig. 1.

Table I. Concentrations of guayulin A and -B in *P. argentatum* and hybrids.

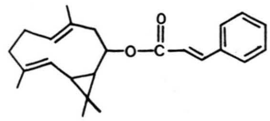
Part of the plant \ Species	*P. argentatum*	*P. argentatum* × *P. tomentosum*	*P. argentatum* × *P. fruticosum*	*P. argentatum* × *P. schotti*
Guayulin-A				
Inflorescence	3.3[a]	0.2	3.6	0.7
Leaf	6.7	1.0	13.8	1.4
Stem	18.6	5.1	32.0	2.6
Root	18.6	3.4	3.8	1.7
Guayulin-B				
Inflorescence	1.9	0.4	0.6	0.3
Leaf	5.6	2.5	3.5	0.8
Stem	2.2	8.2	8.0	1.8
Root	3.2	9.0	1.3	2.3

[a] Concentration is in μmol/g of dry weight and represents the average of 3 different plants concentrations.

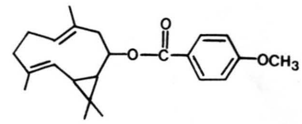
Table II. Distribution of Guayulins in Guayule.

Plant Part \ Compounds	Guayulin A	Guayulin B
Leaf	4.2%[a]	24.7%
Stem	87.6%	66.5%
Root	8.2%	8.8%
206 g[b]	1.22 g[c]	0.18 g

[a] Percentage of total guayulin.
[b] Average dry weight of three whole plants.
[c] Total weight of ester per plant.



Guayulin A



Guayulin B

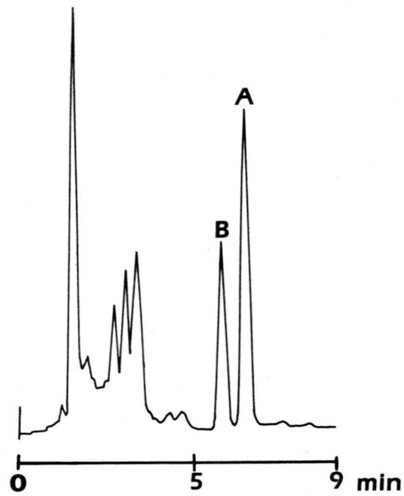


Fig. 1. H.P.L.C. separation of guayulin-A and -B from whole guayule plant (materials worked up as desribed in experimental section). Capacity factor (k') of guayulin B: 1.47 and for guayulin A: 1.91; relative rentention time, $\alpha = 0.77$.

P. argentatum is the only species of the genus that contains significant amount of guayulins A and B. However, in other species of *Parthenium*, the compounds are present in infinitesimal quantities (< 0.05 μmol/g), which suggests that other taxa have the genetic potential to synthesize similar esters. Guayulin A dominates over -B in all parts of the guayule plants. This difference is minimal in the leaves and inflorescence (6.7:6 μmol/g and 3.3:1.9 μmol/g, respectively), but becomes significant in woody parts of plants. While guayulin A exhibits more than two-fold increase in stems and roots, as compared to leaves, guayulin B on the other hand shows a decreasing trend. In order to study the total guayulin content per plant, three guayule plants growing at the Arboretum of the University of California, Irvine were investigated. Plants averaging a weight of 206 g yielded 1.22 g of guayulin A and 0.18 g of guayulin B. The relative distribution of these metabolites is given in Table II, indicating that stems are the main storehouse of guayulins in the total plant. This is supported by the fact that guayulins are the main constituents (more than 10%) of the resin stored in the resin canals and its adjacent cells located in the cortex. This resin also oozes out of the stem at any point of injury.

Analyses of guayulins in the F_1 hybrids of three arborescent species shows two major trends. There is an appreciable increase in guayulin A concentrations in hybrids between guayule and *P. fruticosum*. On the contrary, this concentration is smaller in guayule hybrids with *P. tomentosum* and *P. schottii*. *Parthenium schottii* F_1 hybrids have lesser quantities of guayulin A than *P. tomentosum* hybrids, possibly explained by the fact that the former hybrids are younger than the latter. Dominance of guayulin A over -B, as found in guayule, is exhibited in *P. fruticosum* and *P. schottii* hybrids, however, *P. tomentosum* hybrids showh a revers ratio. This is supported by our earlier investigation of epicuticular alkanes [6] where *P. tomentosum* showed dominant traits in its hybrids with guayule, while it is the opposite in guayule × *P. fruticosum*. Also, this study of resin chemistry suggests dominance of *P. schottii* characters in its hybrids with guayule.

Parthenium argentatum is the only species in the genus and tribe Heliantheae to contain both guayulins and rubber. This species also lacks any sesquiterpene lactones, which are the major constituents of all other members of *Parthenium* (except *P. rollinsianum*). As these compounds are known to possess antimicrobial [7], insecticidal [8], antineoplastic [9] and allergenic [10] properties, an understanding of inheritance of resin components is very crucial in selecting superior rubber producing varieties of *Parthenium* hybrids. It is evident from our present investigations of hybrids of *Parthenium* that the guayulins can be useful genetic markers for breeders. Also, H.P.L.C. analysis is a very sensitive and quick method which could be used by plant breeders to possibly determine the inheritance of resin in the seedlings of guayule and its hybrids.

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