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Note

Detection and quantification of guayulins A and B in *Parthenium argentatum* (guayule) and F_1 hybrids by high-performance liquid chromatography

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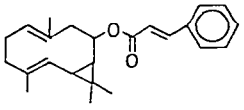
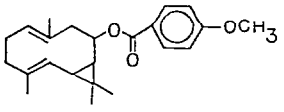
Parthenium argentatum Gray (*Asteraceae*) (common name: guayule) is a small shrub endemic to the Chihuahuan desert of northern Mexico and the south-western parts of Texas, U.S.A., and New Mexico, U.S.A.[1]. Its high rubber content (up to 20% of dry weight) makes it an important agricultural source to partially replace synthetic rubber[2]. Phytochemical studies of *P. argentatum* established the presence of two sesquiterpene phenolic acid esters, germacrene cinnamic acid ester (guayulin A) and germacrene *p*-methoxybenzoic acid ester (guayulin B)[3].



guayulin A



guayulin B

Rodriguez *et al.*[4] recently established that guayulin A is a potent elicitor of contact dermatitis, causing erythremas in sensitized animals at 1.4 nmole. As the cultivation of different strains of *P. argentatum* and crossings with other species of *Parthenium* to increase rubber production is under way, attention should be drawn to the content of the dermatitis elicitors and their inheritance after crossing experiments. High-performance liquid chromatography (HPLC) offers a quick and sensitive method to detect and measure pmole amounts of guayulins A and B in crude plant extracts.

EXPERIMENTAL

Several individual plants of *P. argentatum* and its F_1 hybrids obtained by crossings with *P. tomentosum* var. *stramonium* and *P. fruticosum* var. *trilobatum* were collected during January–March 1981 at the Los Angeles County Arboretum, Arcadia, CA, U.S.A. The plants were separated into leaves and stems, dried, coarsely ground and extracted overnight with distilled acetone. After evaporation, a gummy extract was obtained which was redissolved with acetone for HPLC analysis. Resin

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pouring out of freshly cut stems of *P. argentatum* was collected in vials and dissolved with acetone for HPLC analysis.

A Waters liquid chromatograph was used, equipped with a solvent pump Model 6000A, a universal injector Model U6K and an UV detector Model 440. Detection was achieved at 254 nm. The solvent system was acetonitrile–water (75:25) with a flow-rate of 2.5 ml/min. The HPLC column was Lichrosorb RP-18 (250 × 4.6 mm), pore size 10 μm (Alltech, Los Altos, CA, U.S.A.). Retention times were obtained by measuring the chart (chart speed 0.5 cm/min). Integration of the peak areas was done by height times width at half heights. The quantitative analysis of guayulins A and B in the plant extracts was carried out by using external standards to determine the UV response curves and calculate the linear regression grades. For this purpose guayulins A and B were isolated from *P. argentatum* by means of a SiO_2 column and thin-layer chromatography, solvent system benzene; the guayulins were identified by their ^1H nuclear magnetic resonance spectra[3]. For calculating the capacity factor, k', and the relative retention, α, the equations $k' = (t_R - t_0)/t_0$ and $\alpha = k'_2/k'_1$ were used[5] (t_R = retention time of compound, t_0 = retention time of solvent, k'_2 = capacity factor of compound 2, k'_1 = capacity factor of compound 1).

RESULTS AND DISCUSSION

Leaf and stem tissues of *P. argentatum* and of F_1 hybrids obtained by crossing with other species of *Parthenium* as well as resin samples were screened for their contents of guayulins A and B by HPLC. The guayulins were resolved with baseline separation in the crude plant extracts within ten minutes by using a reversed-phase C_{18} column and applying an isocratic mixture of acetonitrile–water. The retention times and chromatographic parameters k' and α are given in Table I. The detection limit was in the pmole range, allowing reliable quantification of less than gram samples plant material. Quantification was achieved by using the linear regression grades. The UV responses were tested to the nmole range and shown to be linear, with guayulin B being the better chromophor at 254 nm.

TABLE I

RETENTION TIMES (t_R), CAPACITY FACTORS (k') AND RELATIVE RETENTIONS (α) OF GUAYULINS A AND B AND THEIR CONTENTS IN LEAVES, STEMS AND RESIN OF *PARTHENIUM ARGENTATUM*

The amounts given for guayulins A and B are means of several individual plants.

Guayulin-	*t_R (min:sec)*	*k'*	*α*	*Content (μmole/g)*		
				Leaves	*Stems*	*Resin*
B	8:00	4.33	1.20	5	4.4	60
A	9:20	5.22		5.4	13.7	290

The quantitative analysis of the guayulins from leaves, stems and resin samples of *P. argentatum* are given in Table I (for corresponding chromatograms see Fig. 1). The leaf and stem extracts show distinct different patterns. Whereas the amounts of

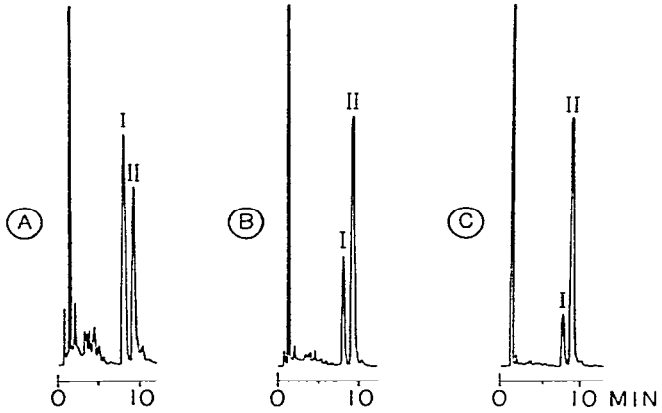

Fig. 1. HPLC resolution of guayulins A (II) and B (I) in (a) leaves; (b) stems; and (c) resin of *Parthenium argentatum* at 0.5 a.u.f.s. For chromatographic conditions see Experimental section.

guayulins A and B are about equal in the leaf tissues (5.4 and 5 μmole/gram dry weight, respectively), guayulin A exhibits more than a two-fold increase (13.7 μmole/gram dry weight) in the stems. Guayulin B is present at slightly lower concentrations (4.4 μmole/gram dry weight) compared to the leaves. The dominance of guayulin A over B is even more significant in the resin that drips out of broken or wounded stems. Ten percent of the resin consists of guayulin A (290 μmole/gram resin) which is five times more than the amount of guayulin B (60 μmole/gram resin).

Analysis of the first filial generations of guayule, obtained by crossing guayule with other species of *Parthenium*, also established the presence of the guayulins. Two hybrid populations of *P. argentatum* × *P. tomentosum* var. *stramonium* and *P. argentatum* × *P. fruticosum* var. *trilobatum* were screened for their contents of guayulins. In all hybrids analyzed the average amounts of guayulins A and B were approximately one fifth to one tenth of the concentrations generally present in *P. argentatum*. The inheritance of the guayulins is in agreement with recent studies on the inheritance of epicuticular alkanes which demonstrated the dominating influence of *P. argentatum* in the F_1 hybrids[6].

Since allergenic reactions of sensitized animals to guayulin A require miniscule amounts of the allergens[4], one can imagine that repeated contact with the resin and its high amounts of guayulins whilst harvesting and processing the plants for rubber extraction could very likely become a serious health hazard. The physiological role of these chemicals within the plant is yet to be determined.

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