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Potent Contact Allergen in the Rubber Plant Guayule (*Parthenium argentatum*)

Abstract. *Chemical and dermatotoxicological investigations of the natural and processed resin of the Mexican rubber plant, guayule* (Parthenium argentatum), *has established the presence of a sesquiterpene cinnamic acid ester (guayulin A) that is a potent elicitor of allergic contact dermatitis in experimental animals. The guayule contact allergen is comparable to the poison ivy skin allergens as an elicitor of dermatitis in sensitized guinea pigs.*

Guayule (*Parthenium argentatum*, Asteraceae) is potentially an economically feasible source of natural rubber. This common shrub, native to the Chihuahuan desert of northern Mexico and southwestern United States, contains *cis*-isoprene rubber (up to 20 percent, dry weight) that is virtually identical to that from the Brazilian rubber tree *Hevea* (Euphorbiaceae) (*1*). In the United States, during World War II, there was an intensive effort to develop commercial rubber production from guayule when *Hevea* rubber imports from Asia stopped, but this program was halted with the discovery that synthetic rubber could be produced inexpensively from petroleum. Today the escalating price of foreign petroleum has made natural rubbers economical, and the development of a guayule rubber and resin industry is under way in Mexico and the United States. We therefore initiated phytochemical and dermatotoxicological studies to determine the potential dermatological hazard of guayule to agricultural and processing-plant employees.

One report of allergic contact dermatitis caused by guayule (*2*) and dermatotoxicological investigations of the genus *Parthenium* (*3*) suggested that a contact allergen was present. We isolated the principal contact allergen from an acetone extract of dried guayule leaves and stems (*4*). The concentrated extract was separated by liquid chromatography over silica gel, and each fraction was assayed for potential to elicit contact dermatitis on guinea pigs sensitized to the crude resin. The sensitization procedure used was the guinea pig maximization test (*5*) for predictive testing in humans. Guinea pigs were given intradermal injections of the crude guayule resin with adjuvant into the shoulder region, followed by topical application of the resin under an occlusive bandage. After 2 weeks, tests for skin hypersensitivity were conducted by applying the separated resin fractions to the shaved skin of the back. For each test, 5 μl of an acetone solution of the test substance was applied to an area of skin 8 mm in diameter. The test sites were not covered and were inspected for eczematous reactions after 24 hours.

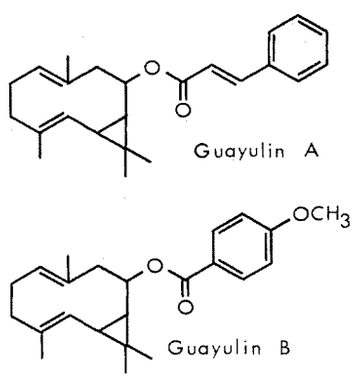

Fig. 1. Structures of guayulin A and guayulin B from *Parthenium argentatum* (guayule).

A single potent elicitor of contact dermatitis was isolated in this manner and purified by recrystallization from hexane. The compound was identified from the ^1H nuclear magnetic resonance (NMR) and infrared spectra (*6*) as the sesquiterpene cinnamic acid ester guayulin A (Fig. 1), which had been previously identified in guayule (*7*). The known sesquiterpene ester of *p*-methoxybenzoic acid, guayulin B (Fig. 1), was also isolated, but it did not exhibit significant allergenic activity. The sensitizing capacity of guayulin A was confirmed by the Freund's complete adjuvant test (*8*); guinea pigs were sensitized to the pure guayulin A and were challenged with serial dilutions of guayulin A in the manner described above. Within 24 hours after application, strong erythemas had appeared on all test sites in response to concentrations as low as 0.010 percent guayulin A (1.4 nmole), with one animal reacting to 0.003 percent (0.5 nmole). The erythemas persisted for 2 weeks. No irritant reactions were observed on a control group of animals sensitized to the adjuvant only.

These experiments show that guayulin A is an extremely potent contact allergen, comparable in potency to the catechol allergens of poison ivy (*Toxicodendron* sp.) (*9*) and to parthenin, a sesquiterpene lactone causing an epidemic skin dermatitis in India (*10*).

Guayulin A is present in both stems and leaves at 0.05 to 0.3 percent, dry weight (*11*), and is one of the major components of the processed resin byproduct obtained from the Saltillo guayule pilot plant in Saltillo, Mexico. Animals sensitized to guayulin A were not sensitive to cinnamic acid (a known contact allergen), to *n*-pentadecyl cinnamic acid ester, or to the sesquiterpene alcohol obtained from base hydrolysis of guayulin A. Nor were the animals sensitive to costunolide, a potent allergenic sesquiterpene lactone similar in structure to the guayulin sesquiterpene moiety. There was only weak cross-reactivity evident with guayulin B (0.42 μmole). These results suggest that delayed hypersensitivity reactions of guayulin A are specific to the intact molecule and are not the result of cleavage of the ester in vivo. The actual mode of action and antigen formation is not

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known for guayulin A, but it is apparent that lipophilicity is important, both for skin penetration and possible insertion into membranes followed by alkylation of skin or serum proteins and lymphocyte recognition (*12*).

The problems that guayulin A presents in a developing guayule industry may be complicated by crossbreeding with other species of *Parthenium* to develop strains of guayule with higher rubber yields (*13*). Guayule readily undergoes interspecific hybridization with *Parthenium incanum* (mariola), a close desert relative, and with *P. tomentosum* var. *stramonium*, an arborescent species common to Sinaloa, Mexico. These two species contain sesquiterpene lactones that are cytotoxic and produce allergic skin reactions in persons initially sensitized to species belonging to the sunflower family (Asteraceae). Preliminary investigations of crosses of *P. tomentosum* var. *stramonium* with guayule indicate the presence of guayulin A and stramonin B, a cytotoxic pseudoguaianolide, in the first filial generation of the experimental hybrids.

Allergic contact dermatitis is a leading occupational health problem today in terms of the number of persons afflicted and probably also in terms of misery (*14*). Guayule-processing facilities can be planned now to minimize worker contact with resins; rubber-processing procedures can be designed to ensure removal of allergens in final products to a safe level; breeding programs can select for strains low in allergen content as well as for high rubber yield. Since widespread cultivation of guayule in marginal arid regions around the world is likely, it would be wise to initiate studies of the potency of guayule allergens on humans and of occupational dermatitis in existing guayule-processing pilot plants.

ELOY RODRIGUEZ*
GARY W. REYNOLDS
JAMES A. THOMPSON
Phytochemistry Laboratory, Department of Developmental and Cell Biology, and *Department of Ecology and Evolutionary Biology, University of California, Irvine 92717*

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6. Guayulin A ^{1}H NMR (90 MHz) spectrum in $CDCl_3$: 0.98 (1H, dd, J = 11 Hz, J = 9 Hz, C-7), 1.10 (3H, s, C-12 or 13), 1.14 (3H, s, C-12 or 13), 1.57 (3H, d, J = 1.5 Hz, C-15), 1.60 (1H, dd, J = 11.5 Hz, J = 9 Hz, C-6), 1.69 (3H, d, J = 1.5 Hz, C-14), 2.1 (5H, m, C-2, 3, and 9'), 2.81 (1H, dd, J = 5.5 Hz, J = 12.5 Hz, C-9), 4.53 (1H, dd, J = 1.5 Hz, J = 11.5 Hz, C-5), 4.92 (1H, dt, J = 5.5 Hz, J = 11 Hz, C-8), 5.15 (1H, m, C-1), 6.44 (1H, d, J = 16 Hz, $-C\underline{H}=CH-C_6H_5$), 7.47 (5H, m, $-C_6H_5$), 7.68 (1H, d, J = 16 Hz, $-CH=C\underline{H}-C_6H_5$).
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11. Guayulin A was assayed by high performance liquid chromatography with detection at 254 nm.
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Metabolic Mapping of the Brain's Response to Visual Stimulation: Studies in Humans

Abstract. *These studies demonstrated increasing glucose metabolic rates in the human primary (PVC) and associative (AVC) visual cortex as the complexity of visual scenes increased. The metabolic response of the AVC increased more rapidly with scene complexity than that of the PVC, indicating the greater involvement of the higher order AVC for complex visual interpretations. Increases in local metabolic activity by as much as a factor of 2 above that of control subjects with eyes closed indicate the wide range and metabolic reserve of the visual cortex.*

Much recent understanding of the anatomical and functional makeup of the visual system has been derived from electrophysiological studies, typically through the use of single cell recordings, such as those carried out by Hubel and Wiesel, who have extensively reviewed the subject (*1*). Some studies in which electrodes have been placed intraoperatively on the cortical surface have also been performed with human subjects (*2, 3*). These and anatomical studies in animals or in humans with lesions in different parts of the visual system have delineated the highly organized structure and function as well as the clear species differences that exist in the visual system.

Scalp electrodes have been used in human electrophysiological studies to measure changes in evoked potentials of the visual cortex during visual stimulation. Although this technique is simple and safe, it has provided very little new information about the visual system because of the poor spatial resolution, difficulty in correlating the evoked potential with cortical neurophysiology, and to some degree the variability of the technique.

Cerebral blood flow, which is considered to provide an index of cerebral function, has been measured in animals to evaluate the visual system's response to stimulation (*4, 5*). Ingvar *et al.* (*6*) injected ^{133}Xe into the carotid artery to study eye movements but not the visual cortex.

Although cerebral blood flow is believed to be normally related to cerebral function, measuring local metabolic rates would provide a more direct evaluation. Human hemispheric metabolism has frequently been studied by the Kety-Schmidt method, but this technique is insensitive to changes in local cerebral function (*7*) and requires jugular vein and arterial catheterization with their associated trauma and risks. An autoradiographic technique for measuring the local cerebral metabolic rate for glucose in animals with 2-[^{14}C]deoxy-D-glucose (DG) has been developed by Sokoloff *et al.* (*8*). DG is a substrate that com-

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