Concise Multigram Purification of Guayulin A from Guayule

Jerome H. Zoeller, Jr.,*,† John P. Wagner,‡ and Gary A. Sulikowski†

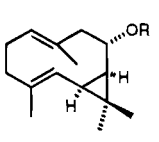
Department of Chemistry, Texas A&M University, College Station, Texas 77843-3255, and Department of Nuclear Engineering, Texas Engineering Extension Service, College Station, Texas 77843-2476

An efficient medium-scale isolation of guayulin A is described. The digestion of defoliated guayule in hot acetone followed by filtration and concentration yields an extract containing resin, low molecular weight guayule rubbers (LGR), and water. Removal of the latter two components affords a transparent green oil. This oil is fractionated by gravity chromatography over neutralized silica gel. A nonpolar concentrate of guayulins A (**1a**) and B (**1b**) first elutes with 10% ethyl acetate in hexane. Further purification by flash chromatography yields pure guayulin A (**1a**) in >98% purity and 0.3% overall yield from the defoliated shrub.

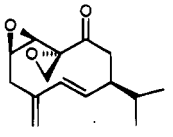
Keywords: *Guayulin A; guayule; purification; phytochemicals*

INTRODUCTION

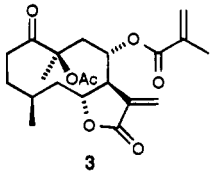
The semidesert shrub guayule (*Parthenium argentatum*) is native to the Chihuahuan desert region of northern Mexico and western Texas. It is a commercial source of natural rubber, second only to the rubber tree (*Hevea brasiliensis*). Since 1888 a number of private and government agencies have initiated programs for the production of natural gum rubber from this resource (Foster and Moore, 1987). Resins comprise about 10% by weight of the dry plant. Potentially useful constituents of the resins are the cinnamate (**1a**) and anisate (**1b**) esters of the sesquiterpene alcohol **1c** (Romo et al., 1970; Watkins et al., 1985), termed guayulin A (**1a**) and guayulin B (**1b**), respectively. The core structure is related to a variety of bioactive natural products including the cockroach pheromone periplanone B (**2**) (Adams



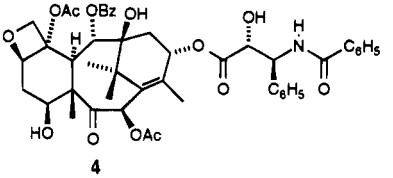
1a R=C(O)CHCHC$_6$H$_5$
1b R=C(O)C$_6$H$_4$(*p*-CH$_3$O)
1c R=H



2



3



4

et al., 1979) and the antineoplastic agent lychnostatin 1 (**3**) (Pettit, 1990) and more subtly to the anticancer agent taxol (**4**). We envisioned the esters **1a** and **1b** could serve as useful starting materials for the chemical synthesis of these biologically important natural products provided enough material could be isolated from the resins. To initiate such synthetic efforts, we would require at least 50 g of either ester with a purity of at least 98% to be available. Herein we describe the isolation of 135 g of guayulin A (**1a**) starting from 100 lb of defoliated shrub.

Schloman (1986) has reported that guayulin A (**1a**) is present at the level of 3600 ppm in the whole shrub and is seasonally variable and dependent on extraction conditions (Schloman et al., 1988). The Gila Indians established stands of the shrub in the early 1980s (Milthorpe and Patterson-Jones, 1991), and the Firestone Tire and Rubber Co. processed the harvest into gum at their facilities in Arizona in the years 1987–1990 (Wagner and Schloman, 1991). Over 8 long tons of rubber was produced. *The principal byproduct of the processing was 8 tons of tar now in the possession of, and under investigation by, the U.S. Department of Agriculture.* The composition of this tar has been studied at Firestone (Schloman, 1983). Our own work (Singh, 1992) showed very early that this tar contains up to 20% by weight of low molecular weight guayule rubbers (LGR). A portion of these rubbers can be removed by digestion in acetone, in which they partially coagulate at room temperature. The esters **1a** and **1b** are detectable in the resins, and the anisate ester **1b** can be isolated in pure form in 0.4% yield from whole Firestone resin. None of the corresponding cinnamate ester **1a** was isolated in pure form. This was surprising to us in view of Schloman's report (Schloman, 1983) that 5–10% of **1a** is isolable from the guayule resins. One reason for this apparent discrepancy is that the guayule was harvested during a period at which the concentration of **1a** was low. With this hypothesis in mind, we reoriented our efforts to the recovery of multigram quantities of esters **1a** and **1b** from fresh plant material.

This paper describes the harvesting, preparation, extraction, and separation of guayule resins into their principal components. The principal components include water, LGR, an unidentified volatile component, polar fractions of the resin, and the nonpolar concentrate which entrains the esters **1a** and **1b**.

MATERIALS AND METHODS

Cautionary Note. Guayulin A is a potent contact allergen (Rodriguez et al., 1981). Guayule resin and the various chromatographic fractions should be treated as potential skin irritants.

Materials. Thin-layer chromatography (TLC) was carried out using 0.25-mm E. Merck precoated silica gel plates. The esters **1a** and **1b** were visualized under ultraviolet light. Flash chromatography was performed with the indicated solvents

† Texas A&M University.
‡ Texas Engineering Extension Service.

0021-8561/94/1442-1647$04.50/0

using silica gel 60 (particle size 0.040–0.063 mm). ^1H and ^{13}C NMR data are reported as δ values relative to tetramethylsilane.

Guayule Preparation and Extraction. Guayule is cultivated at the Texas Agricultural Experiment Station at Fort Stockton. Whole plants of a 3-year old stand of U.S. cultivar 11591 were harvested in early 1993. Clean harvest (January and April, 1993) is defoliated, crudely chopped, ground in a Wiley mill to pass a 4-mm sieve, and air-dried to an equilibrium moisture content of 7%. Resin is expressed from the cells by crushing in a rotating drum flaker spaced at 2 mm. Flaked guayule (30 kg) is distributed among four perforated trays fitted in two 55-gal stainless steel jacketed kettles and covered with 60 gal of acetone (Shell, 99.7%). Extraction proceeds with gentle reflux for an hour with two complete recirculations of miscella. Miscella (50 gal) is drained, the kettles are recharged with acetone, and the second extraction proceeds as before. Solvent is removed from the combined miscella leaving *crude extract* (3600 g) as a viscous turbid green syrup.

Resin Preparation. Water and low molecular weight rubber are removed from the extracts (900 g) by dissolution in ethyl acetate (1800 mL) and washing with brine (2 × 150 mL) in a large separatory funnel. During the process, LGR precipitates, falling as a suspension in the brine, and both are drained away. The organic phase is dried over sodium sulfate, filtered, and concentrated *in vacuo* to yield the resin extract as a transparent green oil (200 g). This process is repeated three times to provide a total yield of 1.1 kg of dry rubber-free resin.

Chromatography. Silica gel (I.D. grade 62, 60 × 200 mesh; 1600 g) is slurried in hexane containing 5% ethyl acetate and 1% triethylamine and packed in a glass column 14 cm in diameter to a depth of 25 cm. Rubber-free resin (400 g) is suspended in 300 mL of hexane–ethyl acetate (1:1), deposited on the top of the packed bed, and eluted with hexane–ethyl acetate (9:1). Fractions were analyzed by thin-layer chromatography (TLC). Esters **1a** and **1b** were detected in the first 7 L of eluant. These fractions were combined and concentrated *in vacuo* to yield 175 g of a yellow oil. A total of 1.1 kg of rubber-free resin yields 320 g of the yellow oil containing a mixture of **1a** and **1b**. This mixture is further purified in 40-g batches by flash chromatography on silica gel (200 × 450 mesh; 250 g) packed in a 10-cm-diameter column as a slurry in hexane (Still et al., 1978) to a depth of 15 cm. One-liter fractions are eluted with hexane. Combination and concentration of fractions 7–13 yields ester **1a** as an oil (15 g). Trituration with hexane precipitates 9 g of guayulin A (**1a**) as white powder: mp 122.0–124.0 °C; ^1H NMR (200 MHz, $CDCl_3$) δ 7.66 (d, J = 16.0 Hz, 1H), 7.50 (m, 2H), 7.40 (m, 3H), 6.43 (d, J = 16.0 Hz, 1H), 5.12 (m, 1H), 4.92 (td, J = 11.1, 5.3 Hz, 1H), 4.52 (d, J = 11.6 Hz, 1H), 2.79 (dd, J = 12.3, 5.3 Hz, 1H), 2.30–1.80 (m, 5H), 1.67 (d, J = 1.4 Hz, 3H), 1.58 (m, 1H), 1.55 (d, J = 1.2 Hz, 3H), 1.13 (s, 3H), 1.08 (s, 3H), 0.97 (dd, J = 11.1, 8.9 Hz, 1H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 166.2, 144.3, 135.8, 134.5, 130.1, 130.0, 128.8, 128.1, 128.0, 125.0, 118.7, 75.4, 42.9, 40.3, 32.9, 28.8, 28.5, 25.2, 21.4, 20.4, 16.5, 15.4. Anal. Calcd for $C_{24}H_{29}O_2$: C, 82.24; H, 8.62. Found: C, 82.01; H, 8.79. Combination and concentration of fractions 14–19 yield a 10-g mixture of **1a** and **1b** as a dark oil.

RESULTS AND DISCUSSION

Evolution of the Process. In a 1951 patent (Meeks et al., 1951) a solvent partitioning technique was described for the isolation of guayulin A, then called "parthenyl cinnamate", from guayule resin. Our own solvent partitioning experiments on the Firestone tars (Singh, 1992) showed the LGR do contaminate nonpolar solvent fractions containing esters **1a** and **1b**. The technique described in the patent is solvent intense and leaves much guayulin A unrecovered, so we used chromatographic methods. LGR interfere with the chromatographic purification of nonpolar concentrates as characterized by irregular flow patterns, channeling,

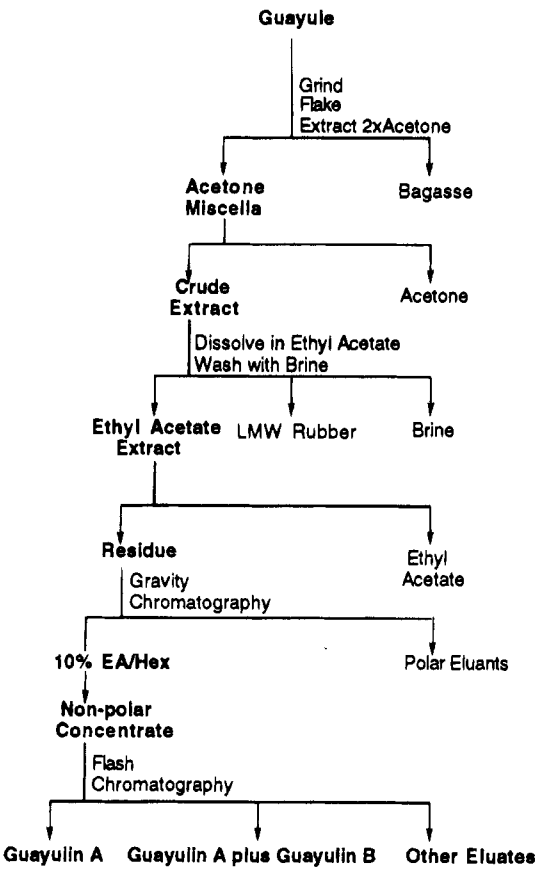


Figure 1. Isolation of guayulin A from guayule.

and plugging of the chromatography column. In addition to this, the rubbers entrain parts of the resin as they coat the adsorbent, leading to lower isolated yields of esters **1a** and **1b**. To first separate high molecular weight rubber, we selected acetone as the extraction solvent. For this reason acetone has been used for the isolation of guayule resins for four decades (Meeks et al., 1951; Eagle, 1981; Wagner et al., 1991), and by workers at Firestone to develop byproducts from the resins (Schloman et al., 1982).

Bench-scale extraction studies were initiated using ground wood covered in acetone at 50 °C for an hour; the extraction was repeated twice. Qualitative analysis of the extracts by thin-layer chromatography indicated that the bulk of the esters **1a** and **1b** was extracted in the first two acetone extract solutions. The third extract is rubber rich, with only traces of esters **1a** and **1b**. In all future experiments the extraction was done only twice, leaving a bagasse entraining the high molecular weight rubbers. Removal of acetone affords a green syrup which partially coagulates on standing at room temperature. Figure 1 outlines the extraction, removal of solvent, and subsequent steps in the full refining process, described under Materials and Methods.

Direct chromatography of the acetone extract led to poor yields of impure esters **1a** and **1b**. Excessive amounts of LGR (~15%) and water (~20%) were identified as a cause of this problem (*vide supra*). Both were efficiently removed by dissolving the extract in ethyl acetate followed by washing with brine and partitioning of the organic layer from the aqueous layer and semisolid precipitated rubbers. Isolation of pure guayulin A (**1a**), and other components, from the rubber-free resin was found to be most efficiently conducted in a two-stage process. First, gravity chromatography served to separate nonpolar components enriched in **1a** and **1b** from

other more polar fractions. Finally, guayulin A (**1a**) was isolated in greater than 98% purity by employing flash chromatography.

In conclusion, we have developed a convenient large-scale isolation of guayulin A (**1b**) from defoliated guayule. In addition to guaylin A (**1b**), the corresponding anisate ester, guayulin B (**1b**), was also isolated, albeit in relatively low yield. The utility of these germacrane esters in the synthesis of biologically important natural products is currently under investigation. Finally, other more polar fractions are under investigation to identify other components with potential commercial utility.

ACKNOWLEDGMENT

Guayule wood was provided generously by Jaroy Moore and Michael Foster of the Texas Agricultural Experiment Station of Fort Stockton. Guayule processing was done at the Food Protein Research Center Pilot Plant at Texas A&M University with the help of Sanjeevan Sanpanthan and Justin Wilson, Plant Manager. This research is sponsored by grants from the U.S. Department of Agriculture (J.P.W.) and the Texas Higher Education Coordinating Board's Advanced Research Program (G.A.S.).

LITERATURE CITED

Adams, M. A.; Nakanishi, K.; Still, W. C.; Varnold, E. V.; Clardy, J.; Persoons, C. J. Sex Pheromone of the American Cockroach: Absolute Configuration of Periplanone-B. *J. Am. Chem. Soc.* **1979**, *101*, 2495–2498.

Eagle, F. A. Guayule. *Rubber Chem. Technol.* **1981**, *51*, 662–684.

Foster, M. A.; Moore, J. Guayule: A rangeland source of natural rubber. *Rangeland* **1987**, *9*, 99–102.

Meeks, J. W.; Banigan, T. F.; Plank, R. W. Process for recovering parthenyl cinnamate and essential oils from guayule resin. U.S. Pat. 2,572,046, 1949, granted Oct 23, 1951.

Milthorpe, P.; Patterson-Jones, J. C.; Whitworth, J. W.; Abel, C.; Miller, W. P. In *Guayule Natural Rubber*; Whitworth, J. W., Whitehead, E. E., Eds.; GMAC-USDA/CSRS: Tucson, AZ, 1991; p 367.

Pettit, G. R.; Herald, D. L.; Cragg, G. M.; Rideout, J. A.; Brown, P. Antineoplastic agents, 178: isolation and structure of lychnostatins 1 and 2 from the south american lychnophora antillana. *J. Nat. Prod.* **1990**, *53*, 382–390.

Rodriguez, E.; Reynolds, G. W.; Thompson, J. A. Potent Contact Allergen in the Rubber Plant Guayule (Parthenium Argintatum). *Science* **1981**, *211*, 1444.

Romo, J.; Romo de Vivar, A.; Ortega, A.; Diaz, E. Guayulins A and B, new sesquiterpenes isolated from guayule. *Rev. Latinam. Quim.* **1970**, *1*, 132–135.

Schloman, W. W., Jr., Ed. "Analysis and utilization of by-products extracted from parthenium argentatum"; Report to U.S. Department of Agriculture; The Goodyear Tire & Rubber Co., 1982.

Schloman, W. W., Jr.; Hively, R. A.; Krishen, A.; Andrews, A. M. Guayule byproduct evaluation: extract characterization *J. Argic. Food Chem.* **1983**, *31*, 873–876.

Schloman, W. W., Jr.; Garrot, D. J.; Ray, D. T.; Bennett, D. J. Seasonal effect on guayule resin compostion *J. Agric. Food Chem.* **1986**, *34*, 177–184.

Schloman, W. W., Jr.; Carlson, D. W.; Hilton, A. S. Guayule extractables: influence of extraction conditions on yield and composition. *Biomass* **1988**, *17*, 239–249.

Singh, M. Bagwa Guayule resin separation and purification M.S. Thesis, Texas A&M University, Department of Nuclear Engineering, Dec 1992.

Still, W. C.; Kahn, M.; Mitra, A. Rapid chromatographic technique for preparative separation with moderate resolution. *J. Org. Chem.* **1978**, *43*, 2923–2925.

Wagner, J. P.; Parma, D. G.; Benedict, C. R. Effect of varying parameters on solvent extraction of natural rubber from guayule. *Polym-Plast. Technol. Eng.* **1991**, *30*, 473–485.

Wagner, J. P.; Schloman, W. W., Jr. In *Guayule Natural Rubber*; Whitworth, J. W., Whitehead, E. E., Eds.; GAMC-USDA/CSRS: Tucson, AZ, 1991; p 261.

Watkins, S. F.; Fronczek, F. R.; Chiari, G.; Reynolds, G. W.; Rodriguez, E. Molecular structure of guayulin A. *J. Nat. Prod.* **1985**, *48*, 631–633.

Received for review February 18, 1994. Accepted June 1, 1994.[⊗]

[⊗] Abstract published in *Advance ACS Abstracts,* July 15, 1994.