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Note

Technique for the identification of small amounts of rubber in biological materials

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Green plants, as well as derived cultured tissues, represent potential sources of biomass for conversion into energy intensive chemicals or direct production of such materials. Guayule is a biomass source that is expected to fill a critical materials shortage: a renewable source of rubber. Because of the potential economic value of guayule, studies are being directed to the development of cultured tissue systems[1,2], as well as genetical breeding programs[3,4], for the evaluation of rubber synthesis. However, there are few techniques available which detect low amounts of rubber quickly and efficiently in small amounts of plant material such as would be obtainable from cultured tissues or small populations of newly developed strains in present pilot studies[5]. Analysis of polymers on a microscale is difficult due primarily to the complexity of the molecules. For example, guayule natural rubber has a molecular weight of *ca.* 10^6, making analysis by gas chromatography (GC) impossible[6]. Yasuda developed an integrated pyrolysis–GC system that decomposes high-molecular-weight polymers into low-molecular-weight volatile compounds characteristic of the analyzed polymer[7]. From his analysis of natural rubber, Yasuda found 2-methyl-1,3-butadiene (isoprene) as the major pyrolysis product. Based on this approach, we developed our own pyrolysis–GC system in order to verify the presence or absence of isoprene, and hence rubber, in guayule materials.

EXPERIMENTAL

Whole plant materials were obtained from defoliated stems of medium-sized (1 ft.) guayule shrubs grown from seed in the Indiana University greenhouse. Stems were dried and crushed with mortar and pestle prior to pyrolysis or benzene extraction.

Sterilization of guayule materials for culture was performed as described earlier[8]. The medium[9] was supplemented with 1 g/l casein hydrolysate and 0.1 g/l myo-inositol. Hormonal additions were 1 mg/ml of both 2,4-D and kinetin. The

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media were adjusted to pH 7.0 and solidified with 0.6% agar. Following growth for 16 weeks, callus tissue was removed from stem explants and dried and crushed.

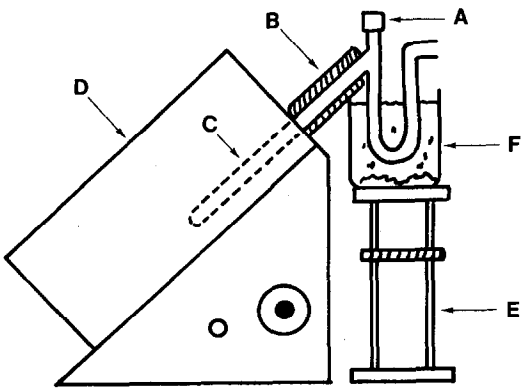
Guayule rubber, obtained from the Research Center of Applied Chemistry in Saltillo, Mexico, was cut into small pieces before pyrolysis.

Whole plant (0.5 g) and tissue culture (0.5 g) materials were extracted in a Bantum-ware micro-soxhlet apparatus with benzene for 20 h. The benzene extracts were transferred by syringe into Pyrex pyrolysis tubes constructed by the Indiana University glass shop (Fig. 1). The solvent was then evaporated by placing the pyrolysis tube in a steam bath for a period of 3–4 days under a stream of nitrogen. Finally, the tube was stoppered and connected to a vacuum pump for 24 h.

Rubber, whole plant, tissue culture callus (0.5 g of each), as well as benzene extract residues of whole plant and callus, were pyrolyzed in a Sargent furnace at 400°C for 10 min. A dry ice–acetone cold trap was used to collect gases and volatile liquids issuing from the pyrolysis tube. Following pyrolysis, the tubes were stoppered and allowed to equilibrate to ambient temperature. Gaseous samples were removed with a 10 μm Pressure-Lok gas syringe and injected into a Varian 3700 gas chromatograph equipped with a flame ionization detector. The column was 5 m × 1/4 in. I.D. copper packed with 20% dibutyl tetrachlorophthalate (DBTCP) on Chromosorb P (60–80 mesh). The temperature was held isothermally at 50°C. Detector and injection port were held at 150°C and 125°C, respectively.

RESULTS AND DISCUSSION

From our investigations we have found the combination of pyrolysis of rubber-containing materials and gas–liquid chromatography provides a qualitative, straightforward determination of rubber presence[10]. When pyrograms are compared



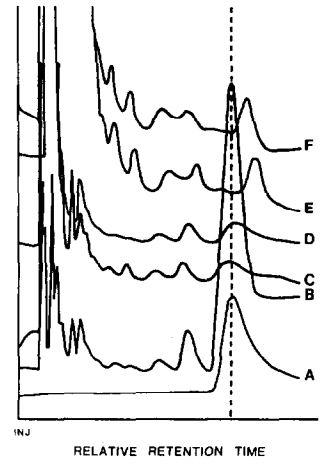


Fig. 1. Pyrolysis unit. A = Serum stopper; B = insulation; C = material to be pyrolyzed; D = Sargent 115 V furnace; E = laboratory jack; F = Dry ice–acetone trap.

Fig. 2. Chromatographic pyrogram of pyrolysates. Composite program of the several samples analyzed for the isoprene moiety. The vertical dashed line represents the retention time for isoprene relative to all samples. Traces: A = isoprene standard; B = guayule rubber; C = guayule whole plant tissue; D = benzene extract of gyayule whole plant tissue; E = guayule tissue cultured callus material; F = benzene extract of guayule tissue cultured callus material.

with standard isoprene GC traces (A), the presence of isoprene becomes readily apparent (Fig. 2).

All pyrograms, with the exception of callus and callus extract pyrograms, share similar profiles consisting of several low-molecular-weight, volatile compounds, and isoprene. As expected, guayule rubber (B) contains large amounts of isoprene. Whole plant material (C), while containing large amounts of C_1–C_4 compounds, has modest amounts of isoprene. The benzene extract of whole plant material (D) also contains modest concentrations of isoprene but with lowered amounts of C_1–C_4 compounds. However, even with a ten-fold scale expansion, no isoprene peak could be detected in tissue culture callus (E) or callus extract (F).

Although we were concerned only with the isoprene peak as positive identification of rubber presence, our pyrograms are also consistent with those of Yasuda[7] who identified a number (10–11) of C_1–C_4 compounds in natural rubber (*Hevea*) pyrograms, including acetylene, methane, ethane, ethylene, propane, and numerous butane derivatives.

As expected, the guayule whole plant and benzene extract pyrograms show lowered isoprene concentrations as compared to pure guayule rubber. The question of rubber production in guayule tissue culture is answered by Fig. 2 (E and F) which indicates the absence of isoprene in whole callus and benzene extract pyrograms. Since no isoprene is detected, rubber was not produced in callus tissue.

Guayule contains no specialized cell type analogous to the laticifer that characterizes *Hevea*. Rather, latex-producing cells found in guayule stems are parenchymatous and do not exude latex when cut. Because both guayule rubber-producing cells and callus tissue are composed of parenchyma-like cells, it was initially postulated that rubber might be produced in culture. However, our results do not support such a hypothesis. Instead, the absence of detectable rubber production raises the question of whether the cells in whole plant and callus are in fact identical. If microstructure analysis of the cells proves them to be identical, the effects of various hormone and precursor media additions on rubber production in callus should be assayed. Should the cells be shown to be morphologically different, further studies must concentrate on inducing the formation of latex producing whole plant type cells in a tissue culture system.

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