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GUTTA-PRODUCING GRASSES

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Key Word Index—Gramineae; grasses; chemotaxonomy; polyisoprenes; gutta.

Thomas Edison examined about 10 species of gymnosperms and 186 species of monocotyledonous angiosperms and found that a few of the latter contained 'rubber' [1]. Although several authors have detected high MW polyisoprenes in fungi, phytochemists generally have persisted in the belief that in *Spermatophyta* only dicotyledonous angiosperms produce these compounds [2]. Edison's results have either been overlooked due to late publication or discounted because his 'rubber' was poorly characterized.

In screening plants for potential as hydrocarbon producing crops [3, 4], we have now analysed 13 Graminae species including 8 of those examined by Edison. Seven species contained no significant amount of the 'hydrocarbon fraction' equivalent to Edison's 'rubber'; one, *Spartina pectinata*, contained a hard wax incompletely extracted by acetone and 5 contained a surprising amount of hydrocarbon, 0.6–1.7 % based on the entire plant's dry weight (Table 1). IR examination indicated that the 'hydrocarbon fraction' of the 5 species was nearly pure *trans*-1,4-polyisoprene.

Purified polymer samples were definitively characterized by ^{13}C NMR and by gel-permeation chromatography. This established unequivocally that these Graminae species synthesize high MW *trans*-1,4-polyisoprene (gutta) (Table 2). While the MWs of the guttas are lower than those of most green-plant rubbers (*cis*-1,4-polyisoprenes) [5], they are high enough for the more crystalline *trans*-polymer to have useful properties [6]. Thus, there is potential practical significance to this discovery in addition to its important scientific implications.

The 'oil fractions' and their unsaponifiable matter were examined by TLC using densitometry [7] to obtain a semiquantitative estimation of the lipid classes present. All the oils from gutta-producing Graminae were similar. However, they were quite different from the whole-plant oils of *S. pectinata* and some other Graminae which do not produce gutta (Fig. 1). They were also very different from the oils of typical rubber-producing species in other plant families, for example the Asclepiadaceae (Fig. 1). The information provided by our screening procedures, i.e. TLC estimation of overall lipid composition along with detailed determination and characterization of hydrocarbon polymers, may be useful for biochemical systematics or chemotaxonomy. Five of the above species can be assigned to a single biochemical group based on their high content of gutta in combination

Table 1. Analysis of grasses

				Acetone extract		Cyclohexane extract	
Genus and species	Month/day collected*	Ash (%)	Crude protein (%)	Polyphenol fraction (%)	Oil fraction (%)	Hydrocarbon fraction (%)	IR characterization of hydrocarbon
Agropyron repens (L.) Besuv.	8/11	11.8	10.8	4.1	2.08	1.72	Polyisoprene
Andropogon scoparius Michx.	10/7	—	—	5.2	1.36	0.15	
Echinochloa crusgalli L.	10/7	—	6.9	2.7	0.91	0.15	
Echinochloa walteri (Pursh.) Nash.	10/7	11.9	13.1	5.1	1.99	0.23	
Elymus canadensis L.	7/28	5.3	6.6	5.2	1.64	1.28	Polyisoprene
Elymus canadensis L.	9/28	6.7	4.4	4.1	0.86	1.48	Polyisoprene
Leersia oryzoides (L.) Sw.	6/29	11.2	14.2	5.6	2.00	0.63	Polyisoprene
Leersia oryzoides (L.) Sw.	10/7	10.3	8.6	6.3	1.21	0.40	Polyisoprene
Leersia virginica Willd.	9/1	13.0	8.4	8.5	1.36	0.67	Polyisoprene
Panicum virgatum L.	10/7	6.1	5.8	3.6	0.63	0.11	
Phalaris canariensis L.	9/1	11.8	8.4	4.9	2.05	1.15	Polyisoprene
Phalaris canariensis L.	9/28	9.7	6.9	4.7	0.92	1.22	Polyisoprene
Phragmites communis Trin.	10/3	6.0	3.8	6.4	1.14	0.27	
Sorgastrum nutans (L.) Nash.	10/7	4.3	2.9	5.7	0.91	0.13	
Spartina pectinata Link.	9/1	5.9	7.3	5.8	2.00	0.25	Wax
Spartina pectinata Link.	9/28	5.1	3.3	4.3	1.43	0.47	Wax
Trioda flava (L.) Smyth	10/7	6.5	6.8	3.0	0.87	0.15	

* Collected in central Illinois, mostly in 1977.

Table 2. MWs of gutta polymers

Plant source	Month/day collected	Weight-average MW $(\bar{M}_w \times 10^{-3})$	MW distribution $(\bar{M}_w/\bar{M}_n)$	^{13}C NMR characterization
Agropyron repens	8/11	111	2.4	*trans*-1,4-polyisoprene
Elymus canadensis	7/28	116	2.4	*trans*-1,4-polyisoprene
Elymus canadensis	9/28	176	2.8	*trans*-1,4-polyisoprene
Leersia virginica	9/1	123	2.7	*trans*-1,4-polyisoprene
Phalaris canariensis	9/1	76.8	2.7	*trans*-1,4-polyisoprene
Phalaris canariensis	9/28	123	3.5	*trans*-1,4-polyisoprene

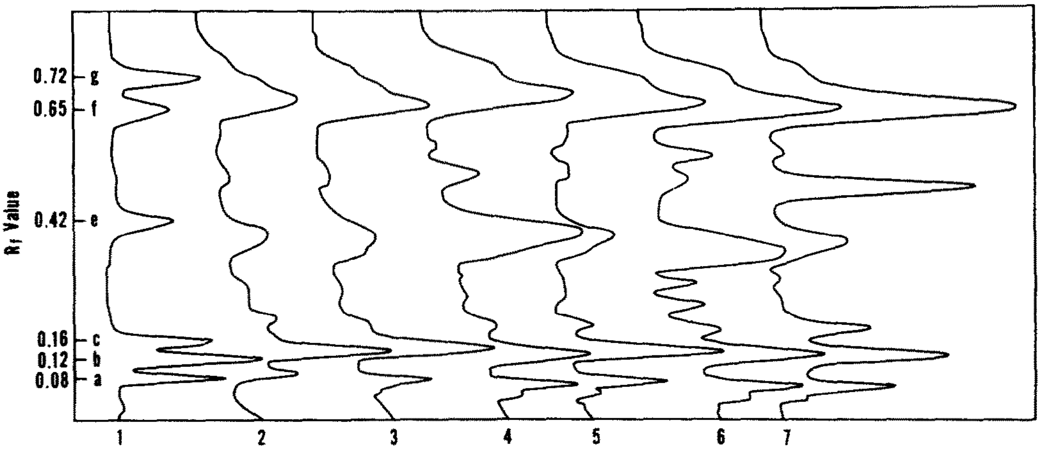

Fig. 1. Comparison of whole-plant oil from gutta-producing Gramineae with those from a non-gutta-producing species and from a rubber-producing Asclepiadaceae, densitometer traces from thin-layer chromatography. (1) standard with 5 μg each sitosterol R_f 0.08 (a), oleyl alcohol R_f 0.12 (b), oleic acid R_f 0.16 (c), triolein R_f 0.42 (d), oleyl laurate R_f 0.65 (e) and sqaulene R_f 0.72 (f); (2) *Agropyron repens*; (3) *Elymus canadensis*; (4) *Leersia virginica*; (5) *Phalaris canariensis*; (6) *Spartina pectinata*; (7) *Ascepias incarnata* oil.

with a characteristic lipid TLC fingerprint.

Polyisoprene synthesis in plants is usually associated with latex, either contained in ducts as in *Hevea brasiliensis* or in thin-walled cells as in *Parthenium argentatum*. The polymer in the live plant is thus stored as a stable emulsion of microscopic particles in an aqueous medium.

Calvin believes that the latex particle, associated surfactants, and an unidentified catalyst are vital to the synthesis of rubber high-polymer by green plants [8]. In his view the plant system functions much like a conventional synthetic emulsion polymerization system with surfactant, catalyst and latex particle size operating to control MW. However, in the above Gramineae, there is no superficial evidence for latex, so the site of polymer genesis and storage is an important matter for further investigation. Perhaps the mechanism for gutta synthesis is entirely different from rubber biogenesis.

The gutta contents of the above wild grasses are comparable to the rubber content of a producing *Hevea* tree on a dry-plant weight basis. Thus, such grasses may be regarded as potential practical sources of gutta. We are investigating the response of *Elymus canadensis* to chemical bioinduction of gutta [9] and expect that yields can be increased several-fold through combined chemical yield stimulation and plant breeding. Gutta would have large-scale applications as both a thermoplastic and a thermosetting resin if it were available at prices competitive with natural rubber and petroleum-derived polymers [6].

EXPERIMENTAL

All plant collection and identification were done by the coauthor I. M. Cull, a botanist. Voucher specimens are being retained at our Center for the present, but probably will be deposited in the U.S. National Herbarium when our screening program is completed.

Experimental details for isolation, identification and characterization of products are given in the authors' earlier publications [3–5, 10].

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THE IDENTIFICATION AND CHARACTERIZATION OF *ORTHO*-DIHYDRIC PHENOLS

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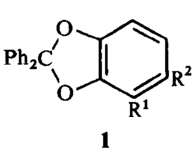
Whilst the benzene-1,2-diol grouping is readily recognized by its chromatographic behaviour on silica impregnated with sodium tetraborate, and the colourations given with iron-III chloride are sometimes diagnostic, a formal proof of this structure requires the preparation of a characteristic derivative. Although dichlorodiphenylmethane reacts readily with benzene-1,2-diol to give the diphenymethylenedioxy (1:1) cyclized product (**1**; $R^1 = R^2 = H$) [1], there have been few examples in natural product chemistry of the use of this reagent for the characterization or identification of *ortho*-dihydric phenols, possibly because the methods employed [2, 3] have been too severe [3] (heating at 170–180°) or unsatisfactory, with the formation of by-products [2, 4]. The structure **2** of the isocoumarin reticulol, since confirmed by synthesis [5], was originally assigned [6] on the basis of a negative reaction with the reagent, a somewhat dangerous assumption since on structure **2** a reaction giving a (1:2) product might have been expected.

As part of a wider investigation of methods for the identification and characterization of *ortho*-dihydric phenols, we have examined the course and scope of this reaction with a number of model compounds, using relatively mild conditions, and have obtained [7] the derivative (**3**, RR = Ph_2C) from fusarentin 6-methyl ether (**3**, R = H).

When equimolar quantities of the reactants were heated at 100°, the desired (1:1) product was obtained in acceptable yield from benzene-1,2-diol in 0.25 hr, from methyl 3,4-dihydroxybenzoate in 1.5 hr, and from methyl 2,3-dihydroxybenzoate in 4 hr. Under milder conditions, more suited to acid-sensitive materials, the product (**1**; $R^1 = R^2 = H$) was obtained after 1 hr heating under reflux in benzene in the presence of potassium carbonate, but methyl 3,4-dihydroxybenzoate required 6 hr, and methyl 2,3-dihydroxybenzoate and fusarentin 6-methyl ether [7] required 18–30 hr heating in toluene. Under these conditions, methyl 2,4-dihydroxybenzoate gave the (1:2) product **4**, but reticulol was recovered unchanged, confirming the earlier observation [6].

The unreliability of the iron-III colour reaction as a diagnostic test is demonstrated by the different results obtained with reticulol and the (±)-3,4-dihydroisocoumarin (**5**), obtained by catalytic hydrogenation of reticulol. Whereas **5** gave the reddish-purple colour characteristic of resorcylic acid derivatives, as has been



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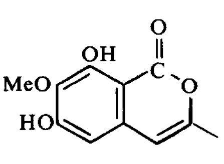

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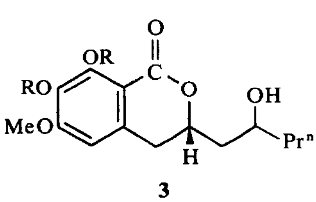

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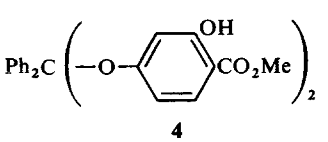

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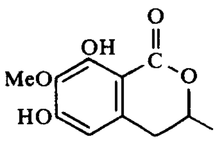

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