

COMPARATIVE ALKANE CHEMISTRY OF *PARTHENIUM ARGENTATUM* (GUAYULE) AND SOME F₁ HYBRIDS

PETER PROKSCH, H. MOHAN BEHL and ELOY RODRIGUEZ

Phytochemical Laboratory, Department of Ecology and Evolutionary Biology, University of California, Irvine, CA 92717, U.S.A

(Received 25 September 1981)

Key Word Index—*Parthenium argentatum*; *P. tomentosum* var. *stramonium*; *P. fruticosum* var. *trilobatum*; Asteraceae; F₁ hybrids; chemotaxonomy; leaf alkanes.

Abstract—The leaf alkanes of *Parthenium argentatum* (guayule), *P. tomentosum* var. *stramonium*, *P. fruticosum* var. *trilobatum*, and the first filial (F₁) generations obtained from crosses with guayule were investigated by GC and mass spectrometry and shown to be useful in chemotaxonomic studies. The identified *n*-alkanes ranged from C₁₉ to C₄₀ with either *n*-C₂₉ or *n*-C₃₁ as the main component. The alkane chemistry of guayule with *n*-C₃₁ being the main component predominated in most of the F₁ hybrids. The presence of *iso*-branched alkanes (C₂₇, C₂₉, C₃₁) in *P. tomentosum* and its hybrids could be detected by GC/MS. These preliminary investigations indicate that epicuticular wax alkanes can be useful in inheritance studies of guayule and its hybrids.

INTRODUCTION

The Mexican rubber plant guayule (*Parthenium argentatum* Gray) is presently being considered as an economically feasible source of natural rubber[1]. Guayule is common to the Chihuahuan desert of northern Mexico and to the south-western United States and is reported to contain up to 20% by dry wt of rubber and a plethora of sesquiterpene phenolic esters and triterpenes[2]. Recent chemical and dermatotoxicological studies of the natural and processed resin of guayule have established the presence of a sesquiterpene cinnamic acid ester that is a potent elicitor of allergic contact dermatitis in experimental animals[3]. A guayule breeding program has been established at the Los Angeles County Arboretum with guayule, a small shrub of ca 50 cm height, crossed with more robust and taller species of *Parthenium*. The purpose of the breeding program is to produce hybrids that synthesize large quantities of rubber combined with higher biomass production and increased plant pathogen resistance factors. We have initiated phytochemical studies of the parents and F₁ hybrids in order to better understand inheritance of hydrocarbons and resistant factors. In this present study we report the epicuticular leaf alkane chemistry of F₁ hybrids from *P. argentatum* Gray × *P. tomentosum* DC. var. *stramonium* and *P. argentatum* × *P. fruticosum* Less var. *trilobatum*.

RESULTS AND DISCUSSION

Leaf alkanes are well established as suitable plant constituents for chemotaxonomical studies[4–8]. We analysed the alkane patterns of *P. argentatum*, *P.*

tomentosum var. *stramonium*, *P. fruticosum* var. *trilobatum* and F₁ hybrids obtained by crossing *P. argentatum* with the latter two species. Table 1 shows the alkane profiles of the parent species and the F₁-hybrids. The *n*-alkanes range from C₁₉ to C₄₀, with the main differences expressed in the varying percentages of the predominating components *n*-C₂₉ and *n*-C₃₁. The alkane pattern of guayule with *n*-C₃₁ (36.6%) predominating over *n*-C₂₉ (23.5%) shows significant differences in comparison to *P. tomentosum* and *P. fruticosum*. The *n*-alkane C₂₉ (37.5 and 31%, respectively) predominates over C₃₁ (33.7% and 27.5%, respectively) in *P. tomentosum* and *P. fruticosum*. Whereas the profiles of the *n*-alkanes of the latter two species show similarities, clear differentiation is indicated by the presence of 2-methyl branched alkanes (*iso*-alkanes) in *P. tomentosum*. *Iso*-C₂₇, C₂₉, and C₃₁ were identified by the enlarged relative intensities of the [M – 15]⁺ and [M – 43]⁺ fragments in their mass spectra corresponding to the cleavage on both sides of the 2-methyl side chain [9, 10]. The relative percentages for the three species given in Table 1 are the means of several parallel examinations from different collections for each species. The s.d.s range from 1 to 2.5%. The F₁ hybrid populations of *P. argentatum* × *P. tomentosum* and *P. argentatum* × *P. fruticosum* are rather heterogeneous in their phenology. According to the differences, four individual plants of each hybrid population were screened for leaf alkanes (Table 1). Each individual plant was given a number that refers to the hybrid population and locality at the Los Angeles County Arboretum and to the voucher specimens deposited at the UCI Herbarium. Of all hybrids analysed, only

Table 1. Leaf alkane profiles as percentages from species of *Parthenium* and *F₁* hybrids

Carbon No.	<i>P. tomentosum</i> , var. <i>stramonium</i>		<i>P. tomentosum</i> var. <i>stramonium</i> × <i>P. argentatum</i> *		<i>P. argentatum</i>		<i>P. argentatum</i> × <i>P. fruticosum</i> var. <i>trilobatum</i> *		<i>P. fruticosum</i> , var. <i>trilobatum</i>
	78-397-1	78-394-15	78-392-4	78-394-27	<i>P. argentatum</i>	78-388-11	78-388-9	78-384-22	79-148-54
19-22	†		†	†	†	†	†	†	†
23	†		†	†	0.7	1.9	1.0	†	0.8
24	†	0.4	†	†	0.4	†	0.7	†	†
25	0.3	0.9	0.6	0.5	2.2	1.6	1.9	1.7	1.7
26	†	†	†	†	0.6	†	1.0	†	†
iso-27	†	†	†	†	+	+	+	+	+
27	5.2	6.0	6.2	6.0	9.5	5.9	6.9	5.2	4.8
28	1.1	0.7	1.0	1.4	1.8	2.2	2.1	†	1.2
iso-29	2.7	1.4	†	†	+	+	+	+	+
29	37.5	33.4	33.6	28.5	23.5	30.5	27.9	26.7	26.2
30	2.3	1.7	2.2	2.2	4.3	1.2	3.5	1.7	2.4
iso-31	0.6	0.8	†	†	+	+	+	+	+
31	33.7	36.1	35.0	35.0	36.6	32.4	24.5	38.4	39.6
32	1.1	0.9	0.8	2.4	2.7	†	1.4	†	1.4
33	2.9	4.4	3.2	4.7	4.5	1.6	2.8	4.9	4.5
34	3.0	2.1	2.3	4.9	3.2	3.3	3.5	2.3	2.2
35	†	†	0.4	†	0.4	†	1.4	2.9	1.4
36	5.9	5.9	10.7	8.4	5.1	11.8	11.7	9.3	6.7
37	†	†	†	†	†	†	†	†	†
38	3.7	4.4	4.0	6.0	4.5	7.6	9.7	6.9	7.1
40	†	†	†	†	†	†	†	†	†

†os. refer to designations at the Los Angeles County Arboretum and to voucher specimens at the UCI Herbarium.

0.1%.

Values given for the three species are means of three independent samplings. For each sampling 20-30 different plants were harvested and screened for leaf alkanes. Values for the *F₁* hybrids are for individual plants which show distinct different morphological features referred to by the number given.

one hybrid (No. 78-397-1) was a natural hybrid. The inheritance from both parents *P. argentatum* and *P. tomentosum* is expressed by the presence of iso-branched alkanes inherited from *P. tomentosum* that could be detected in all of its hybrids and by significant domination of *n*-C₃₁ as characteristic for *P. argentatum* in two of the hybrids studied. Among the four individuals, No. 78-394-15 seems to be closest related to *P. argentatum* although *n*-C₂₉ (33.4%) and *n*-C₃₁ (43.5%) are present in higher percentages. The alkane chemistry of *P. argentatum* is also clearly expressed in the *n*-alkane pattern of No. 78-394-27 the difference between *n*-C₂₉ (28.5%) and *n*-C₃₁ (35.0%) being smaller than that for *P. argentatum* but still characteristic within the above given standard deviations. The other two individuals of this hybrid population, (Nos. 78-397-1 and 78-392-4) show similar percentages of *n*-C₂₉ (33.8 and 33.6%, respectively) and *n*-C₃₁ (36.1 and 35.0%, respectively). In the range of s.d.s given, the alkane profiles of these two plants resemble more the pattern of *P. tomentosum* than that of *P. argentatum*.

Clear differences in the alkane profiles were also observed in the individuals of the F₁ hybrid population of *P. argentatum* × *P. fruticosum*. Hybrid Nos. 78-384-22 and 79-148-54 contain *n*-C₃₁ (38.4 and 39.6%, respectively) and *n*-C₂₉ (26.7 and 26.2%, respectively). Even the relative percentages of these two alkanes are similar to the ones found in *P. argentatum*. A stronger influence of *P. fruticosum* is present in hybrid No. 78-388-11, with hardly any quantitative differences noted between *n*-C₂₉ (30.5%) and *n*-C₃₁ (32.4%). The strongest resemblance to the alkane profile of *P. fruticosum* is notable in hybrid No. 78-388-9 with *n*-C₂₉ (27.9%) predominating over *n*-C₃₁ (24.5%).

It is apparent from this study that the chemical analysis of leaf epicuticular wax alkanes can be an important tool in surveying and identifying hybrid populations which sometimes are difficult to distinguish by morphological features.

EXPERIMENTAL

The species of *Parthenium* and F₁ hybrids were cultivated at the Los Angeles County Arboretum, Arcadia, California. Plant material was collected during November and Decem-

ber 1980. Two hybrid populations, *P. argentatum* × *P. tomentosum* and *P. argentatum* × *P. fruticosum* were analysed, with individuals that showed distinct morphological differences investigated for leaf alkanes. Detailed descriptions of the hybrids will be reported elsewhere [Behl, H. M., in prepn]. Epicuticular waxes were extracted by dipping fresh leaves into Et₂O. The Et₂O extract was taken to dryness and redissolved in *n*-C₅H₁₂. Fractionation of the crude extract was achieved by Si gel CC (Type 60, Merck, 70–230 mesh). Alkanes were eluted with *n*-C₅H₁₂ [4]. GC carried out on a FID instrument. The column (stainless steel, 1.8 m × 0.3 cm id) was packed with 3% OV101 on Chromosorb W-HP. Injector temp. was 250°, detector temp. 300°. A linear temp. program from 200 to 290° at 4°/min was used. The upper temp. was maintained for 15 min. The N₂ flow rate was 30 ml/min. Identification of *n*-alkanes achieved by comparison with standard alkanes and by GC/MS. GC/EI-MS were recorded at 70 eV.

Acknowledgements—We thank Henry Yokoyama, USDA, Los Angeles, for kindly recording the MS and Paul-Gerhard Gülz, Botanical Institute of the University of Cologne, West Germany, for *n*-alkane standards. We are indebted to the Los Angeles County Arboretum, Arcadia, Los Angeles, for providing the plant material. The financial support of the National Science Foundation, the UCI Graduate Division and the Fulbright Commission is acknowledged.

REFERENCES

1. La Breque, M. (1980) *Mosaic* 11, 30.
2. Rodriguez, E. (1977) *Biochem. Syst. Ecol.* 5, 207.
3. Rodriguez, E., Reynolds, G. and Thompson, J. A. (1980) *Science* 211, 1444.
4. Königs, R. and Gülz, P.-G. (1974) *Z. Pflanzenphysiol.* 72, 237.
5. Herbin, G. A. and Robins, P. (1968) *Phytochemistry* 7, 239.
6. Herbin, G. A. and Robins, P. A. (1969) *Phytochemistry* 8, 2985.
7. Engel, R. and Gülz, P.-G. (1976) *Z. Pflanzenphysiol.* 79, 401.
8. Köhlen, L. and Gülz, P.-G. (1976) *Z. Pflanzenphysiol.* 77, 99.
9. Eglinton, G., Hamilton, R. J., Kelly, W. B. and Reed, R. I. (1966) *Phytochemistry* 5, 1349.
10. Gülz, P.-G. (1968) *Phytochemistry* 7, 1009.