

rye inhibitor (Granum, 1978) and pearl millet inhibitor (Chandrasekher and Pattabiraman, 1983) which are known to be made up of two subunits but linked by disulfide bridges.

Like the pearl millet inhibitor (Chandrasekher and Pattabiraman, 1983) the *Setaria* inhibitor was found to be relatively heat labile and susceptible to proteolytic digestion. In view of this, the inhibitor may not be of much nutritional significance. An interesting property indicated here is the capability of the inhibitor to protect the target amylase from inactivation under acid conditions.

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**Registry No.**  $\alpha$ -Amylase, 9000-90-2; Blue-Sepharose, 66456-82-4.

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## Composition of Leaf Oils in the Genus *Parthenium* L. Compositae

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Essential leaf oils were steam distilled from seven *Parthenium* species: *P. tomentosum*, *P. fruticosum* var. *trilobatum*, *P. schottii*, *P. incanum*, *P. argentatum*, *P. confertum* var. *lyratum*, *P. hysterothorus*. Only *P. argentatum* and *P. confertum* contain bornyl acetate, thus providing perhaps a link between the woody section *Parthenichaeta* and the herbaceous section *Argyrochaeta*.

While the development of synthetic rubber alleviated our country's total dependence on foreign sources of natural rubber, it increased our dependence on petrochemicals which are in a state of irreversible depletion. There are some applications where only natural rubber can be used, and this keeps the U. S. dependent on foreign sources for this critical raw material. Because the prices of both petroleum and natural rubber are subject to influences beyond our control, any developments which will partially free us from this dependence will be of benefit to our national interests. The 1983 Critical Agricultural Materials Act (H. R. 2733) provided an impetus for continuing the development of a domestic source of natural rubber. It will have the advantage of being a renewable resource and diminish our demand on the depleting fossil petrochemicals (Calvin, 1976).

The identification of any useful byproducts will enhance the chances for the successful commercialization of the production of natural rubber from guayule, *Parthenium argentatum* A. Gray.

Present breeding programs are directed at selecting guayule lines for increased rubber content, producing hybrids with increased biomass, and also hybrids with a greater cold tolerance to extend the range of cultivations in the US.

In guayule, the rubber is found in the stems, so the leaves are initially stripped off and discarded. Volatile oils can be distilled from this waste. The components in the oils must first be identified before any assessment of their value can be made. The possibility of increasing desirable oil constituents by breeding should not be overlooked. Interest in the volatile oils from *P. argentatum* dates back to Alexander (1911), Haagen-Smit and Siu (1944), and Scora and Kumamoto (1979). Dominguez et al. (1971) identified limonene and  $\alpha$ -pinene from the steam distillate of fresh material from *P. incanum* H.B.K. We have recently reported on the identification of bornyl acetate from *P. argentatum* and *P. confertum* var. *lyratum* A. Gray (Kumamoto and Scora, 1984).

We wish to report on our results of a survey of leaf oils from seven species in the genus *Parthenium*. The taxa examined here are *P. tomentosum* DC, *P. fruticosum* Rollins, *P. schottii* Greenm., *P. incanum* H.B.K., *P. argentatum* A. Gray, *P. confertum* var. *lyratum* A. Gray, and *P. hysterothorus* L.

#### EXPERIMENTAL PROCEDURE

Plants of seven species were grown in a University of California, Riverside, lathhouse. Samples (500 g) of healthy, mature leaves were harvested between 0830 and 1000 h and immediately ground and steam distilled under an atmosphere of carbon dioxide, and the oils collected in a Clevenger trap. A Varian 1520 GC with a thermal conductivity detector and a linear temperature program of 1

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Table I<sup>a</sup>

	elution time, s	<i>P.</i> <i>tomentosum</i>	<i>P.</i> <i>fruticosum</i>	<i>P.</i> <i>schottii</i>	<i>P.</i> <i>incanum</i>	<i>P.</i> <i>argentatum</i>	<i>P.</i> <i>confertum</i>	<i>P.</i> <i>hysterophorus</i>
yield of oil, mL/1000 g FW		0.45	0.13	1.05	0.90	1.48	0.44	0.33
oil component, % composition								
methanol	396	1.4	-	+	+	0.3	+	-
ethanol	609	-	0.3	-	-	0.2	+	-
C <sub>7</sub> H <sub>12</sub>	891	-	-	-	-	-	0.3	-
$\alpha$ -pinene	1293	2.2	3.2	35.5	17.5	23.8	10.4	+
thujene	1490	6.2	-	-	3.0	-	-	-
camphene	1795	0.8	0.1	0.1	0.4	1.7	-	0.1
$\beta$ -pinene	2301	21.9	2.2	9.5	10.7	19.5	1.8	0.1
sabinene	2640	3.4	1.4	1.1	3.6	9.3	0.5	0.2
$\beta$ -myrcene	3336	1.7	1.7	0.6	1.8	3.6	1.9	4.7
$\alpha$ -terpinene	4092	1.1	-	-	4.2	-	0.1	0.3
limonene	4216	4.2	0.5	0.2	9.4	8.4	-	0.2
terpinolene	4494	0.7	4.3	-	3.5	13.2	-	-
1,8-cineole	4800	1.5	-	-	3.0	-	-	-
unknown	5337	-	-	-	-	-	4.9	-
$\beta$ -ocimene	5500	4.7	-	1.8	-	3.0	-	0.3
ocimene	5880	1.8	1.6	1.1	3.3	0.3	3.0	6.1
$\rho$ -cymene	6347	0.2	-	-	0.1	0.4	-	3.1
$\beta$ -phellandrene		-	-	-	-	0.6	-	-
hexanol	6800	0.2	0.1	-	0.2	0.3	-	-
<i>cis</i> -3-hexenol	7344	0.4	0.1	0.2	-	0.4	-	-
unknown	8109	-	-	-	-	-	2.0	1.9
unknown	8925	0.8	0.4	0.5	-	-	-	-
unknown	9135	1.1	1.9	0.2	0.8	0.4	-	-
linalool	9200	1.2	0.4	0.4	1.0	0.1	2.0	4.6
bornylacetate		-	-	-	-	5.9	0.6	-
caryophyllene	9701	18.8	26.3	18.9	3.9	+	5.1	6.9
humulene	10213	+	2.1	0.1	0.5	0.6	-	0.4
terpinene-4-ol	10230	0.5	0.6	5.7	1.6	0.4	0.3	0.6
unknown	10405	1.4	4.0	-	6.7	1.7	7.7	6.7
(valencene)	10508	5.8	19.2	+	9.9	2.0	51.1	35.8
other unidentified components, eluting later		18.0	29.6	24.1	14.9	3.9	8.2	28.0

<sup>a</sup>+ = trace amounts <0.1%. - = not detected.

°C/min from 50 to 150 °C, with a 304.8 × 0.635 cm O.D. stainless steel column packed with 20% LAC 2R446 on 60–80 mesh Chromosorb W, and 60 mL/min of helium carrier gas was used to separate the components. A Finnigan 1015 S/L quadrupole mass spectrometer with a Varian 1400 GC and controlled with a pdp 8/m system/150 computer was used to obtain mass spectra for the structural confirmation of the oil components from *P. incanum*, *P. argentatum*, and *P. schottii*.

## RESULTS AND DISCUSSION

*Parthenium argentatum*, the most advanced species of the subgenus *Parthenichaeta*, had the highest yield of oil [1.48 mL/1000 g fresh weight (FW)] as well as the highest amount of rubber. It also has the highest fraction of leaf oil in the monoterpene hydrocarbons at 83.8%. This is followed by *P. incanum* (a low rubber producer) with 60.4%, then *P. schottii* with 49.9%, followed closely by *P. tomentosum* with 48.9%, followed by *P. confertum* with 22.6%, followed by *P. hysterophorus* with 15.1%, and finally *P. fruticosum* with 15.0% of their leaf oils in the monoterpene hydrocarbon fraction.

*P. schottii* has the highest fraction of  $\alpha$ -pinene with 35.5%, as well as the highest amount with 0.37 mL/1000 g FW. It is closely followed by *P. argentatum* with 23.8% and 0.35 mL/1000 g FW. Next to *P. hysterophorus*, which had only a trace amount of  $\alpha$ -pinene, it is *P. tomentosum* which had the lowest fraction of  $\alpha$ -pinene with 2.2%, as well as a low amount of 0.01 mL/1000 g FW. The lowest amount, however, is exhibited by *P. fruticosum* with 0.004 mL/1000 g FW.

*P. tomentosum* had the highest fraction of  $\beta$ -pinene at 21.9% and the amount of 0.1 mL/1000 g FW. It is *P. argentatum* which had the highest amount of  $\beta$ -pinene

with 0.29 mL/1000 gFW and a percentage of 19.5. Next to *P. hysterophorus* having only 0.1% of  $\beta$ -pinene and 0.0003 mL/1000 g FW, it is *P. confertum* which had the lowest fraction of 1.8%, and the next lowest amount of 0.008 mL/1000 g FW.

Sabinene is present in the highest fraction (9.3%) in *P. argentatum*, as well as in the highest amount of 0.14 mL/1000 g FW, followed by *P. incanum* with 3.6% and the amount of 0.03 mL/1000 g FW. It is lowest in *P. hysterophorus* with 0.2% and 0.0007 mL/1000 g FW.

All species tested have caryophyllene, but *P. argentatum* has only minute traces. The major component in *P. argentatum* eluting in this region was identified from its mass spectrum as bornyl acetate.

The identification of the monoterpene constituents in the steam-distilled leaf oils from different *Parthenium* species has been interpreted as evidence of their relationships at a molecular level. We generally assume that the presence of the same monoterpene products in different species suggests that a common biosynthetic pathway is present in each. The presence of these enzyme systems that produce the same products suggests a close or analogous genetic basis for a species relationship.

We accept, as previous authors have proposed, that *P. tomentosum* is the most primitive member of the woody *Parthenichaeta* subgenus, and that the main line of advancement developed toward the herbaceous species, section *Argyrochaeta*. *Parthenium fruticosum* developed early in this advancement scheme with *P. schottii* and *P. cineraceum* branching off from the main line at an early stage.

The most specialized woody *Parthenichaeta* are the rubber yielding desert species *P. incanum*, *P. argentatum*, and *P. rollinsianum* (Rodríguez, 1975). While *P. incanum*

represents an apomictic complex, *P. argentatum* is an outcrossing species with significant genetic diversity.

*P. argentatum* is the only species in the Parthenochaeta which contains bornyl acetate. We have found only one other species, in the herbaceous section, *Argyrochaeta*, *P. confertum* var. *lyratum*, which also contained bornyl acetate. This suggests the presence of a common biosynthetic pathway and infers a close or analogous genetic basis so that these two can serve as a bridge between the two sections, Parthenochaeta and *Argyrochaeta*.

*P. hysterothorus* is the most advanced herbaceous species. It is an aggressive weed which successfully spread northeast into Texas. It is highly allergenic (Towers et al., 1977; Loukar et al., 1974). One of us contracted an itchy rash on the hand which spread discontinuously to other places and was acute for about three weeks. Lingering residual effects persisted for over a year.

The absence of bornyl acetate is perhaps puzzling because it arose in *P. argentatum*, persisted in *P. confertum*, and then vanished in *P. hysterothorus*. From a presumed chromosome base number of  $x = 6$ , the woody species of the Parthenochaeta acquired  $n = 18$ , as well as *P. confertum* of *Argyrochaeta* (Rollins, 1950).

*P. hysterothorus* has lost one chromosome ( $n = 17$ ) and this lends credence to the plausibility that the absence of bornyl acetate may be associated with the missing chromosome.

**Abbreviations used:** FW, fresh weight.

**Registry No.** Methanol, 67-56-1; ethanol, 64-17-5;  $\alpha$ -pinene, 80-56-8; thujene, 58037-87-9; camphene, 79-92-5;  $\beta$ -pinene, 127-91-3; sabinene, 3387-41-5;  $\beta$ -myrcene, 123-35-3;  $\alpha$ -terpinene, 99-86-5; limonene, 138-86-3; terpinolene, 586-62-9; 1,8-cineole, 470-82-6;  $\beta$ -ocimene, 13877-91-3;  $\alpha$ -ocimene, 502-99-8; *p*-cymene, 535-77-3;  $\beta$ -phellandrene, 555-10-2; hexanol, 111-27-3; *cis*-3-hexenol, 928-96-1; linalool, 78-70-6; bornyl acetate, 76-49-3; caryophyllene, 87-44-5; humulene, 6753-98-6; terpinen-4-ol, 562-74-3.

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## Fusarin C: Structure Determination by Natural Abundance $^{13}\text{C}$ - $^{13}\text{C}$ Coupling and Deuterium-Induced $^{13}\text{C}$ Shifts

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The structure of fusarin C, a relatively potent mutagen produced by the common fungus *Fusarium moniliforme*, was determined principally by analysis of NMR data. The carbon backbone was established by analysis of natural abundance  $^{13}\text{C}$ - $^{13}\text{C}$  coupling patterns and positions of oxygen and nitrogen substitution were established by analysis of deuterium-induced shifts in resonances of certain carbon atoms. Stereochemistries of double bonds were established by analysis of vicinal  $^{13}\text{C}$ - $^1\text{H}$  and vicinal  $^1\text{H}$ - $^1\text{H}$  couplings.

Fusarin C is the major bacterial mutagen produced by *Fusarium moniliforme* grown on corn under laboratory and field conditions (Wiebe and Bjeldanes, 1981; Gelderblom et al., 1983; Gaddamidi and Bjeldanes, 1984). Corn infected with *F. moniliforme* caused cancer in laboratory animals (Yang, 1980) and has been associated with cancer in human populations in Africa and China (Marasas et al., 1981; Li et al., 1980). The structure elucidation of fusarin C depended primarily on long-range  $^1\text{H}$ - $^{13}\text{C}$  and natural abundance  $^{13}\text{C}$ - $^{13}\text{C}$  coupling as determined from a two-dimensional double-quantum coherence experiment (Mareci and Freeman, 1982; Bax et al., 1981). Analyses

of deuterium-induced shifts in appropriate  $^{13}\text{C}$  resonances provided a basis for assignments of oxygen and nitrogen substitution (Pfeffer et al., 1979).

Fusarin C (1) is a yellow oil  $[\alpha]_D^{25} +47.04$  (2.0% in MeOH) isolated from *F. moniliforme* grown on cracked corn (Wiebe and Bjeldanes, 1981). The mutagenic fraction was passed sequentially through columns of LH-20 (MeOH), silica gel (MeOH- $\text{CH}_2\text{Cl}_2$ ), and LH-20 (MeOH). Purification was completed by semipreparative chromatography on a low-pressure silica gel column (Si60, size B, 2.0% MeOH in  $\text{CH}_2\text{Cl}_2$ ). The molecular formula,  $\text{C}_{23}\text{H}_{29}\text{NO}_7$ , was established by combustion analysis and by high-resolution mass spectral analysis ( $m/e$  found 431.1953, required 431.1993). The UV absorptions at 365 nm ( $\epsilon$  25 655) and 265 nm ( $\epsilon$  8250) are characteristic of a substituted polyene. The IR spectrum showed intense absorption bands for OH and NH at 3300-3600  $\text{cm}^{-1}$  and for  $\gamma$ -lactam, ester, and conjugated ketone carbonyls at

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