

Figure 2. Regression equation and best-fit curve for integrator response to tebuthiuron concentration.

Table I. Recovery of Tebuthiuron and Monuron When 10  $\mu\text{g}$  of Each Herbicide Is Added to Soil Samples and Extracted under Conditions of Shaking, Sonicating, or a Combination of Shaking and Sonicating

herbicide	extraction method	% recovery
tebuthiuron	soil: <sup>a</sup> shake	77 $\pm$ 4
	sonicate	85 $\pm$ 5
	shake and sonicate	86 $\pm$ 5
monuron	ether partition	95 $\pm$ 3
	soil: <sup>a</sup> shake	80 $\pm$ 4
	sonicate	84 $\pm$ 5
	shake and sonicate	86 $\pm$ 5
	ether partition	95 $\pm$ 4

<sup>a</sup> Soil extracts were also partitioned into diethyl ether preceding quantification.

conducted under conditions of shaking, sonicating, or a combination of both (Table I). Generally, the total extraction procedure consistently accounted for more than 86% of both herbicides added to soil samples and 5% of the herbicide loss was accounted for as being lost during partitioning the herbicides into diethyl ether (Table I). The ether-partitioning procedure was considered necessary, as a sample-cleaning step, in preparation for introduction into the HPLC system. Although 86% recovery is low, much of the tebuthiuron loss is adjusted when using the

IS to quantify tebuthiuron in soil samples. Also, use of an IS adjusts for variations in extraction efficiency and instrument sensitivity.

Soil CEC, organic matter content, and clay content did not influence the extractability of tebuthiuron or monuron from soil (data not included). The average tebuthiuron recovery from 15 soils was  $98 \pm 0.05\%$ , when using the IS to adjust for extraction losses, and was not significantly different between soils. Also, the RCR for tebuthiuron compared to that for monuron was  $0.85 \pm 0.03$  and was not significantly different between soils.

This procedure of quantifying tebuthiuron was determined to be simple, inexpensive, accurate, and repetitive. Also, the sensitivity range of this method is adequate for most field studies with tebuthiuron. Assuming an application rate of 2.2 kg/ha tebuthiuron and an even distribution of the applied tebuthiuron within the top 15 cm of soil, the tebuthiuron concentration in the soil would be 1.0  $\mu\text{g/g}$  of soil. Therefore, 25-g soil samples would allow for the detection of a 10-fold decrease in tebuthiuron concentration in soil samples.

Registry No. Tebuthiuron, 34014-18-1; monuron, 150-68-5.

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## Identification of Bornyl Acetate in the Leaf Oil from *Parthenium argentatum* A. Gray

Bornyl acetate has been identified by gas chromatography and mass spectroscopy as a component of the leaf oil from *Parthenium argentatum*. Mechanistic schemes for the mass fragmentation of camphor and bornyl acetate are proposed.

Prospects for the foreseeable future (when the fossil fuel supply gets diminished) will require direct utilization of photochemical energy for the production of hydrocarbon fuels. Byproduct processes such as the conversion of phytomass to fuel, as well as to fodder, is presently under vigorous investigation. Calvin (1976) has already suggested that the direct synthesis of hydrocarbon fuel in some plants offers a tremendous potential for the efficient conversion of carbon dioxide to fuel using the free energy obtained from sunlight.

The optimization of latex production by plants has produced in *Hevea brasiliensis* Muell. Arg. an efficient conversion of  $\text{CO}_2$  to rubber by sunlight. Chemical bioinduction of isoprenoids has recently been under investigation by Yokoyama (1977) in *Parthenium argentatum* A. Gray, guayule, a plant under current development as a domestic source of natural rubber. The chemical investigation of dried guayule stem oils dates back to Alexander (1911), who identified  $\alpha$ -pinene, and Haagen-Smit and Siu (1944), who identified  $\alpha$ - and  $\beta$ -pinene, di-

Table I. Mass Spectra for Camphor,  $M_r$  152

Wiley	
126, 95, 81, 39, 69, 108, 152	
100, 97, 79, 48, 43, 39, 29	
Sadtlar	
126, 95, 41, 81, 39, 69, 108, 83, 55, 26	
100, 97, 71, 71, 46, 44, 39, 37, 36, 35	
Quadrupole	
95, 81, 55, 108, 69, 93, 67, 41, 109, 152, 26, 126	
100, 75, 52, 45, 43, 42, 41, 35, 33, 15, 11, 0	

pentene, cadinene, and probably phellandral, in fresh guayule plants. Zutshi et al. (1976) reported on the inhibitory activity of the essential oils, presumably from the leaves of *Parthenium hysterophorus* A. Gray against four bacteria. Dominguez et al. (1971) identified limonene and  $\alpha$ -pinene in the steam distillate of fresh material from *Parthenium incanum* H.B.K., and Scora and Kumamoto (1979) identified the major monoterpenes in the steam distillate from *P. argentatum* leaves.

The successful production of natural rubber will provide two important benefits to the national interest. First, some domestic economic control and competition on the supply of a critical raw material will be welcomed. Second, guayule offers an additional bonus in that unlike other rubber plants, it should be feasible to store rubber in the plant and stockpile crude rubber in the field. The plant itself is sufficiently hardy to survive under minimal conditions.

The development of any useful byproduct from *P. argentatum* will have a favorable economic impact on the development of the cultivation of natural rubber. The first step in the byproduct area is the identification of any specialty chemicals that are present in *P. argentatum*.

In the processing of guayule rubber, the leaves represent a nuisance waste problem. These leaves carry oils that can be collected by steam distillation. We report on the identification of bornyl acetate, which elutes in the sesquiterpene region of the gas chromatogram.

#### EXPERIMENTAL PROCEDURE

The methods used for the examination of the steam-distilled leaf oils have been previously reported (Scora and Kumamoto, 1979). In addition, a 60 m  $\times$  0.249 mm i.d. fused silica capillary column with a 0.25- $\mu$ m film of DB-5 was used in a Packard Model 7831 gas chromatograph with a hydrogen flame ionization detector. A sample of pure *l*-bornyl acetate was obtained from Fritzsche Brothers, Inc., New York.

#### RESULTS

Table I lists the mass spectra for camphor taken from the literature, as well as the spectrum obtained with a quadrupole mass spectrometer. The sorted array for the quadrupole spectrum no. 304 from the volatile leaf oil from *P. argentatum* is 43 (100), 95 (73), 41 (41), 93 (42), 121 (22), 55 (21), 69 (18), 67 (17), 80, 39, 15 (14), 79 (13), 136, 108, 29 (12), 92, 81, 27 (11), 53 (10), 154 (3), and 196 (0.2).

#### DISCUSSION

In general, there is a reasonable agreement between mass spectra obtained on a quadrupole spectrometer at 70 eV and those in the published literature from magnet instruments. Camphor appears to be a notable exception where the fragmentation pattern listed for this monoterpene in the Wiley and Sadtlar files are strikingly different from its quadrupole spectrum. The principal ion at 126 amu is absent in the quadrupole spectrum! The identification and the mechanistic pathways for obtaining the ions present in the camphor spectrum will have a direct

Table II. Fragmentation of Camphor

126	95	81	55	108	69	93
$C_{10}H_{16}O$	$C_7H_{11}$	$C_6H_9$	$C_4H_7$	$C_8H_{12}$	$C_5H_9$	$C_7H_9$
$C_9H_{14}O$	$C_6H_7O$	$C_5H_5O$	$C_3H_3O$	$C_7H_8O$	$C_4H_5O$	$C_6H_5O$
$C_9H_2O$						
$C_8H_{14}O$						
67	41	109	71	83	152	26
$C_5H_7$	$C_3H_5$	$C_9H$	$C_5H_{11}$	$C_6H_{11}$	$C_{12}H_8$	$C_2H_2$
$C_4H_3O$	$C_2HO$	$C_8H_{13}$	$C_4H_7O$	$C_5H_7O$	$C_{11}H_2O$	
		$C_7H_9O$			$C_{11}H_4O$	
					$C_{10}H_{16}O$	

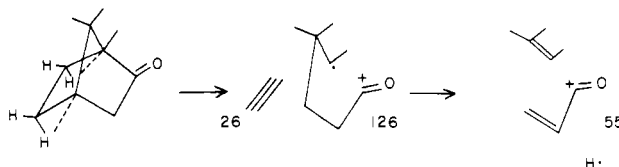


Figure 1. Three center rearrangements of camphor.

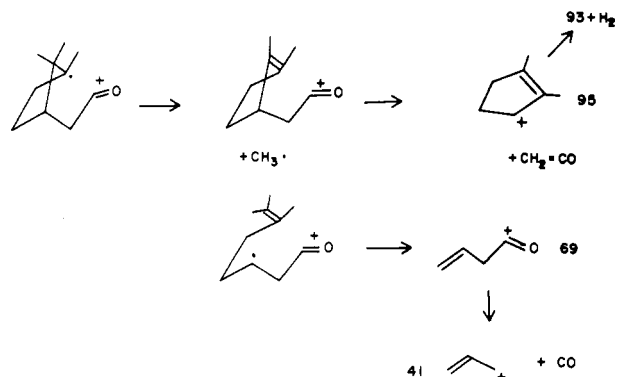


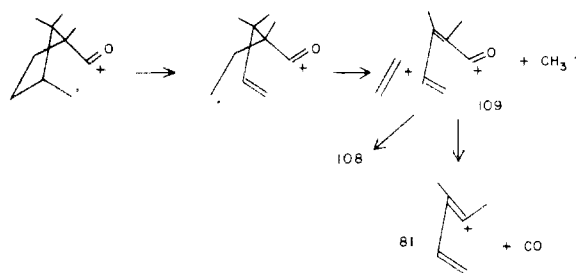
Figure 2. Mechanistic pathway to ions 95, 93, 69, and 41 from camphor.

application in understanding the spectrum of bornyl acetate.

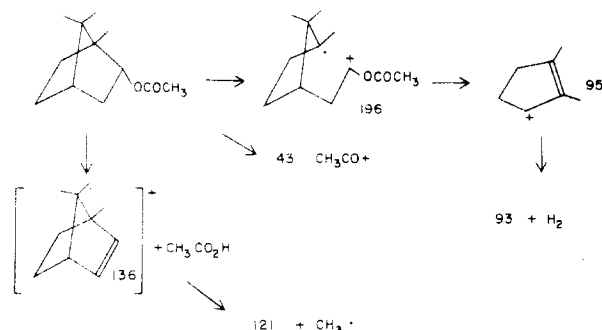
If we start with camphor,  $C_{10}H_{16}O$ , there are a very limited number of ways that carbon, hydrogen, and oxygen can combine to produce the observed mass fragments. These are listed in Table II. Unlikely cleavages to mass 126 would be from a loss of the oxygen atom and 10 hydrogen atoms, the loss of a carbon atom and oxygen atom with the addition of two hydrogens, or the loss of a carbon atom and 14 hydrogen atoms. On the other hand, the cleavage of  $C_2H_2$  would lead directly to  $C_8H_{14}O$ . We propose that the cleavage of  $C_2H_2$  arises from two (probably simultaneous) three-center rearrangements (Figure 1).

The presence of mass 26 in the quadrupole spectrum suggests that the cleavage to mass 126 is occurring but in the quadrupole instrument it does not survive, and we propose that it cleaves again to a smaller fragment at mass 55,  $C_3H_3O$ . This appears to be the only direct pathway to mass 55, and when mass 126 is present, mass 55 is much less abundant.

There are only two possible combinations for mass 95. Since borneol, isoborneol, and bornyl acetate have the same principal ion, it seems unlikely that when the oxygen function were in the reduced form, it would not give a corresponding increase in mass due to the addition of hydrogen. Therefore, a mechanistic scheme to produce  $C_7H_{11}$  is proposed and shown in Figure 2. One of the two expected ions from carbonyl cleavage followed by the loss of methyl and ketene would give us mass 95. The loss of an additional two hydrogens would result in further ion stabilization and give a cyclopentadienonium ion at mass 93. The alternative  $\beta$ -cleavage of the bridge followed by



**Figure 3.** Mechanistic pathway to mass 109, 108, and 81 from camphor.



**Figure 4.** Mechanistic pathway to ions 95, 93, 43, 136, and 121 from bornyl acetate.

the loss of a  $C_6$  radical fragment would produce mass 69, which upon loss of CO would also account for mass 41. The other expected ion from a carbonyl cleavage could lead to the loss of ethylene and then methyl and produce mass 109. The loss of a hydrogen would give rise to mass 108, or the loss of CO would produce mass 81 (Figure 3). We have accounted for all the main fragments in the quadrupole spectrum for camphor with the exception of mass 67. This could arise from the loss of  $H_2$  from mass 69 in Figure 3, but it is also possible that a  $C_5H_7$  fragment is present.

The observation of a molecule ion at mass 196 was crucial in the identification of bornyl acetate. A mechanistic pathway accounting for ions 43, 95, 93, 136, 121, and

196 is presented in Figure 4. The deduction of a possible structure led us directly to the confirmation of elution times with a packed polar column and the nonpolar capillary column, as well as a match of the mass spectrum with reference material.

Of the seven species examined, *Parthenium tomentosum* Rollins, *Parthenium fruticosum* Rollins, *Parthenium schottii* Greenm., *P. incanum* H.B.K., *P. argentatum* A. Gray, *Parthenium confertum* var. *lyratum* A. Gray, and *P. hysterophorus* A. Gray, only *P. argentatum* and *P. confertum* contain bornyl acetate. This provides a chemotaxonomic reason for suggesting a closer ancestral relationship between *P. argentatum*, an advanced member of the subgenus *Parthenichaeta*, and *P. confertum*, a lower member of the subgenus *Argyrochaeta*, in the species relationships proposed by Rollins (1950).

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**Registry No.** Bornyl acetate, 76-49-3.

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