

Composition of Essential Oil from *Proboscidea louisianica* (Martyniaceae)

Michael S. Riffle¹, George R. Waller² and Don S. Murray¹

Departments of Agronomy¹ and Biochemistry²,

Oklahoma Agricultural Experiment Station, Oklahoma State University, Stillwater, OK 74078-0454

Richard P. Sgaramello

International Flavors and Fragrances, 800 Rose Lane, Union Beach, NJ 07735

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The essential oil was collected from mature plants of *Proboscidea louisianica* by neutral or acidic steam distillation and analyzed with a Capillary Gas Chromatography/Mass Spectrometry/Data System (CGC/MS/DS). The MS-50 mass spectrometry of the essential oils required approximately 140 min and produced 3,500 spectra for each sample. The following compounds were identified from the normal essential oil of the roots: vanillin, perillyl acetate, δ -cadinene, α -bisabolol, traxolide, 2-methyl-1,4-naphthoquinone, 1-hydroxy-2(-3)-hydroxymethyl-9,10-anthracenedione, and palmitic acid, with small amounts of 6-methyl-5-hepten-2-one and piperitenone. In the steam distillate of the HCl-treated pods the compounds were vanillin, phenethyl alcohol, *p*-cymen-9-ol, a trimethylcyclohexanone, lauric (dodecanoic) and palmitic (hexadecanoic) acids, and tentatively 2-ethylbenzimidazole.

INTRODUCTION

Proboscidea louisianica (Mill.) Thell., a member of the family Martyniaceae, is known as devil's claw or, sometimes, as unicorn-plant or ram's horn. It is a spreading annual with stems up to 80 cm long and large, entire, opposite leaves up to 30 cm wide. The entire plant is covered with glandular hairs or trichomes, each tipped by a droplet of oil which makes the plant odoriferous and oily to sight and touch (1). The fruit is a drupaceous dehiscent capsule with a stout fruit body up to 100 mm long. The fruit body is terminated by an incurved beak that is longer than wide; at maturity the outer exocarp dries and falls away and the endocarp beak splits to form a 2-horned claw (2).

A white-seeded devil's-claw is sometimes cultivated in the Western U. S.; the young fruit may be pickled for food or the mature fruit may be used as ornaments or as basketry fiber (3). Devil's-claw is native to the southwestern U. S. and northern Mexico, and is the most widely distributed member of its family, ranging from Florida to California, north to Minnesota and south to Mexico (4).

There are no data on the biochemistry of trichomes in devil's-claw plants. Ghosh and Beal (5) found that the major fatty acid components of the seed oil are: linoleic acid (C_{18:2}), \approx 60%; oleic acid (C_{18:1}), \approx 30%; palmitic acid (C_{16:0}), the major saturated acid, \approx 6%. Traces of eight other fatty acids were found. The sterols in the oil were β -sitosterol (80%), campesterol (15%), and four others in minor quantities. The tocopherols in the seeds was comprised of τ -tocopherol (50-60%), α -tocopherol (15%), and δ -tocopherol (30%). The oil content of the seed totaled about 40%; the oil resembles soybean oil.

The objectives of this research were to isolate and identify some of the constituents of devil's-claw essential oil. Preliminary accounts of this work have been presented (6, 7). An account of the activity of this essential oil and six of its components as allelochemical agents on cotton and wheat has been published (8).

METHODS

Steam distillation: Nine mature devil's claw plants were collected from the Perkins, OK, Agronomy Research Station on September 10, 1986, and partitioned into three categories: roots, stems and leaves, and pods. Separately, the material of each category was cut into 3-6 cm pieces, loaded into a 6-liter round-bottom flask, and steam-distilled for 5 h. The apparatus was an all-glass assembly with Teflon stopcocks and sleeves. The condensate (approximately 3 liters) was saturated with NaCl, and extracted with 1 liter of diethyl ether, three times; the extract was

dried over anhydrous Na_2SO_4 and evaporated to dryness under nitrogen. The residue left after distillation was acidified with 2 N HCl to a pH of 0.8 and steam-treated for 5 h as was done in processing *Nepeta cataria* essential oil (9). The devil's-claw condensate was processed in the same way as that from the normal distillation. After the ether extract was freed of solvent, a viscous, dark yellow to light brown oil with a very acrid odor remained.

Capillary gas chromatography: The initial analyses were carried out on a Hewlett Packard Model 5880 gas chromatograph containing a flame ionization detector and an OV-1 fused silica column, 50 mx0.32 mm. The samples were taken up in ether and analyzed by using 1.5- μl injections with a splitter ratio of 25:1, the oven initially at 50 °C and programmed at 2 °C/min to 225 °C and held there for 60 min, and a helium flow of 0.5 ml/min.

Capillary gas chromatography/mass spectrometry/data system analysis: Two of the samples, the normal distillate of the roots and the distillate of the acidified pods, were subjected to gas chromatography and mass spectrometry. The Kratos MS-50 mass spectrometer was equipped with a Varian Model 3700 gas chromatograph containing an OV-1 fused silica column, 50 mx0.32 mm. The samples were analyzed by using 1.0- μl injection with the splitter turned off, oven at 50 °C, programmed at 2 °C/min to 225 °C and held there for 60 min. The helium flow was 0.5 ml/min. The data were acquired and analyzed with a modified Kratos DS-55 data system (10). Identifications were based on comparison with known spectra, visual interpretation of the fragmentation patterns, and the IFF proprietary indices of the elution program (similar to Kovacs indices).

RESULTS

The CGC/MS/DS analysis of the essential oils required approximately 140 min and 3,500 spectra were taken. Data for the essential oil from only roots (normal plant material) and pods (HCl-treated material) are reported here. Similar results were found with stems and leaves. The identified compounds are listed in Table 1. The sensitivity was increased for the total ion current monitoring to show more detail in Figs. 1 and 2. For the essential oil from the roots (Fig. 1), scans 500 to 1000 (Fig. 1A), showed peaks corresponding to *p*-vinylphenol (Fig. 3A), piperitenone (Fig. 3B), and vanillin (Fig. 3C). From scans 1000 to 1500 (Fig. 1B), peaks identified were those of 2-methyl-1,4-naphthoquinone, ionol, 1,3,5-tri-*tert*-butylbenzene, and α -bisabolol (Fig. 3D). Ionol may be a natural compound but it is also used as a preservative present in the ether used. From scans 1500 to 2000 (Fig. 1C), palmitic (hexadecanoic) acid, δ -cadinene (Fig. 3E), and traxolide (Fig. 3F) were identified. From scan 2000 to 2500 (Fig. 1D), (1-hydroxy-2(-3)-hydroxymethyl)-9, 10-anthracenedione was identified. Trace amounts of 6-methyl-5-hepten-2-one were found (data not shown). The remaining compounds were mostly terpenes, terpenoids, and other hydrocarbons, and were not further identified.

In the steam distillate from HCl-treated pods (Fig. 2), scans 500 to 1000 (Fig. 2A), showed peaks corresponding to phenethyl alcohol, a trimethylcyclohexanone, *p*-cymen-9-ol (Fig. 3G), and vanillin. The compound eluting at scan 665 with a molecular mass of 142 remains unidentified. From scans 1000 to 1500 (Fig. 2B), compounds identified were 2-ethylbenzimidazole (tentatively) and lauric (dodecanoic) acid. An isomeric C_{12} acid eluted at scan 1265. From scans 1500 to 2000 (Fig. 2C), palmitic (hexadecanoic) acid was identified. A peak at scan 1650 with a molecular mass of 232 is unidentified. A very large peak of an unknown hydrocarbon with high molecular mass was present at scan 1880. The remaining peaks mostly repre-

TABLE 1. Presence and relative amounts of compounds found in the essential oil of *Proboscidea louisianica* by MS-50 CGC/MS/DS.

Compound	Plant Part	Relative Amount
(1-hydroxy-2(-3)-hydroxymethyl)-9,10-anthracenedione	root	high
α -bisabolol	root	mod ^a
δ -cadinene	root	mod ^a
<i>p</i> -cymen-9-ol	pod	low
hexadecanoic acid	both ^a	high
6-methyl-5-hepten-2-one	root	low
2-methyl-1,4-naphthoquinone	root	mod
perillyl acetate	root	low
phenethyl alcohol	pod	low
piperitenone	root	low
traxolide	root	mod
a trimethylcyclohexanone	pod	high
vanillin	both ^a	high

^a both = root and pod; mod = moderate.

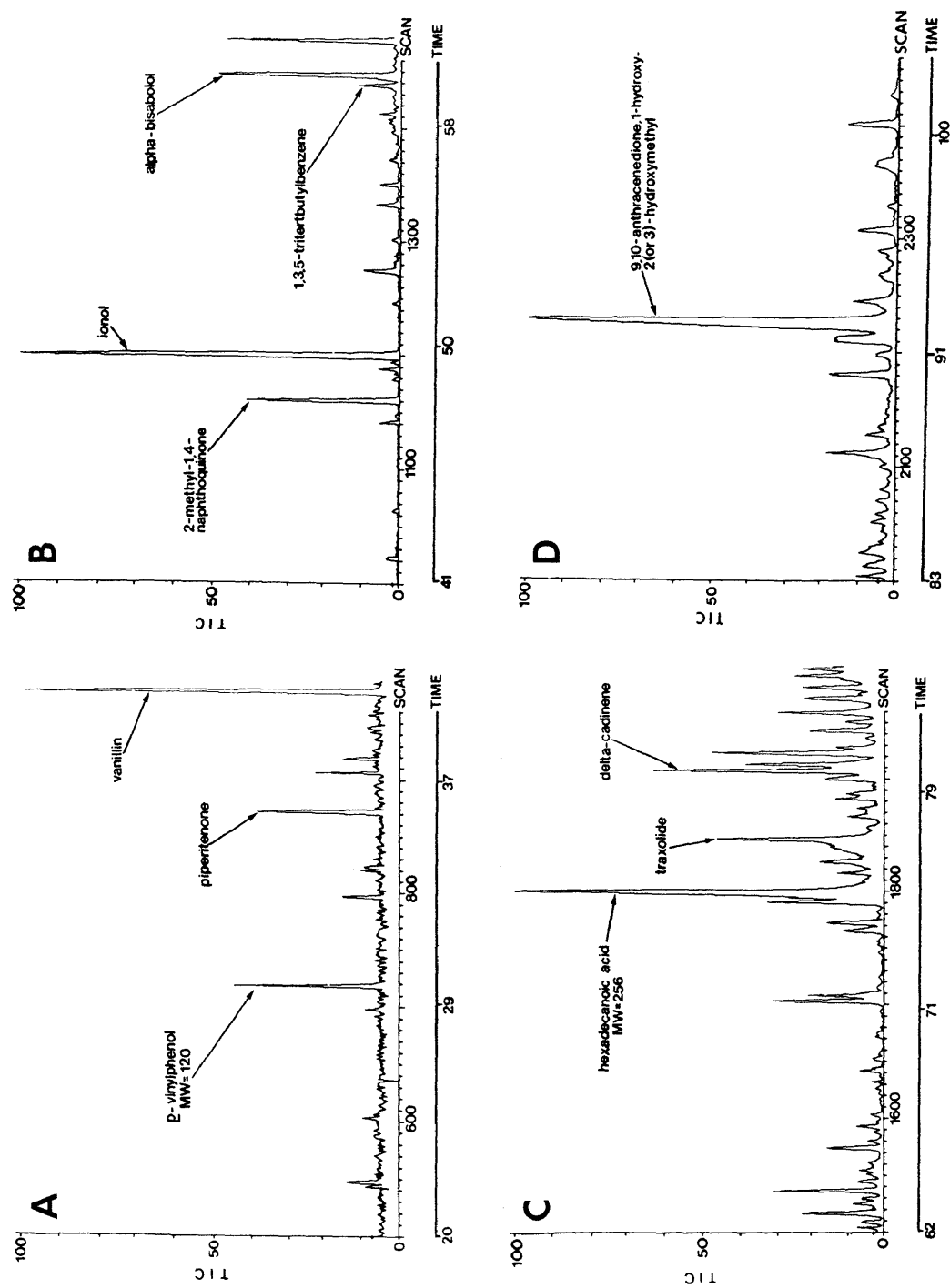


Figure 1. Partially reconstituted total-ion-current (TIC) chromatograms of normal essential oil of *Proboscidea louisianica*. The sensitivity is higher than that shown in Figure 2. (A) Scans No. 500–1000; (B) Scans No. 1000–1500; (C) Scans No. 1500–2000; (D) Scans No. 2000–2500.

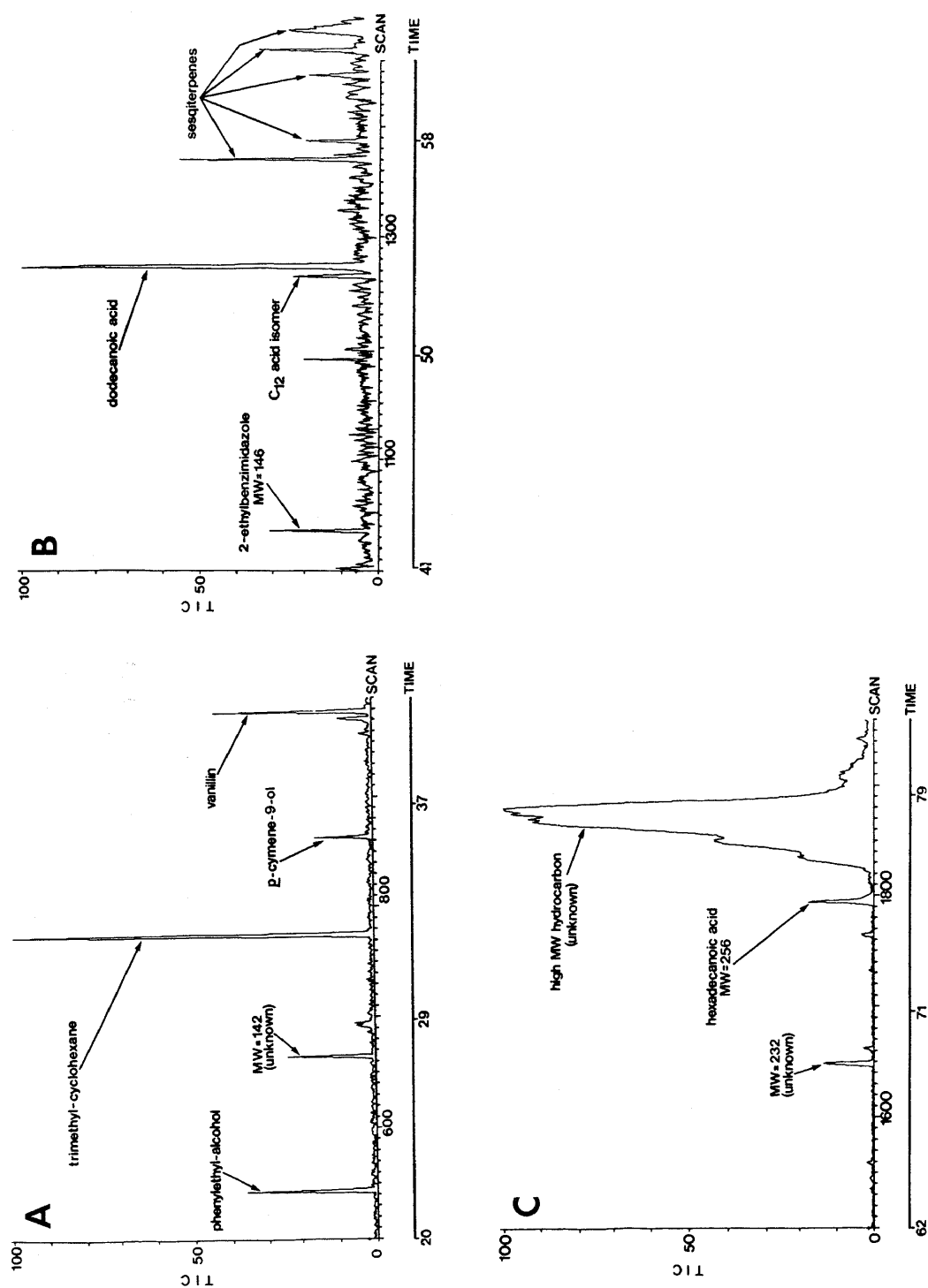


Figure 2. Partially reconstituted total-ion-current (TIC) chromatograms of HCl-treated essential oil of *Proboscidea louisianica*. (A) Scans No. 500–1000; (B) Scans No. 1000–1500; (C) Scans No. 1500–2000.

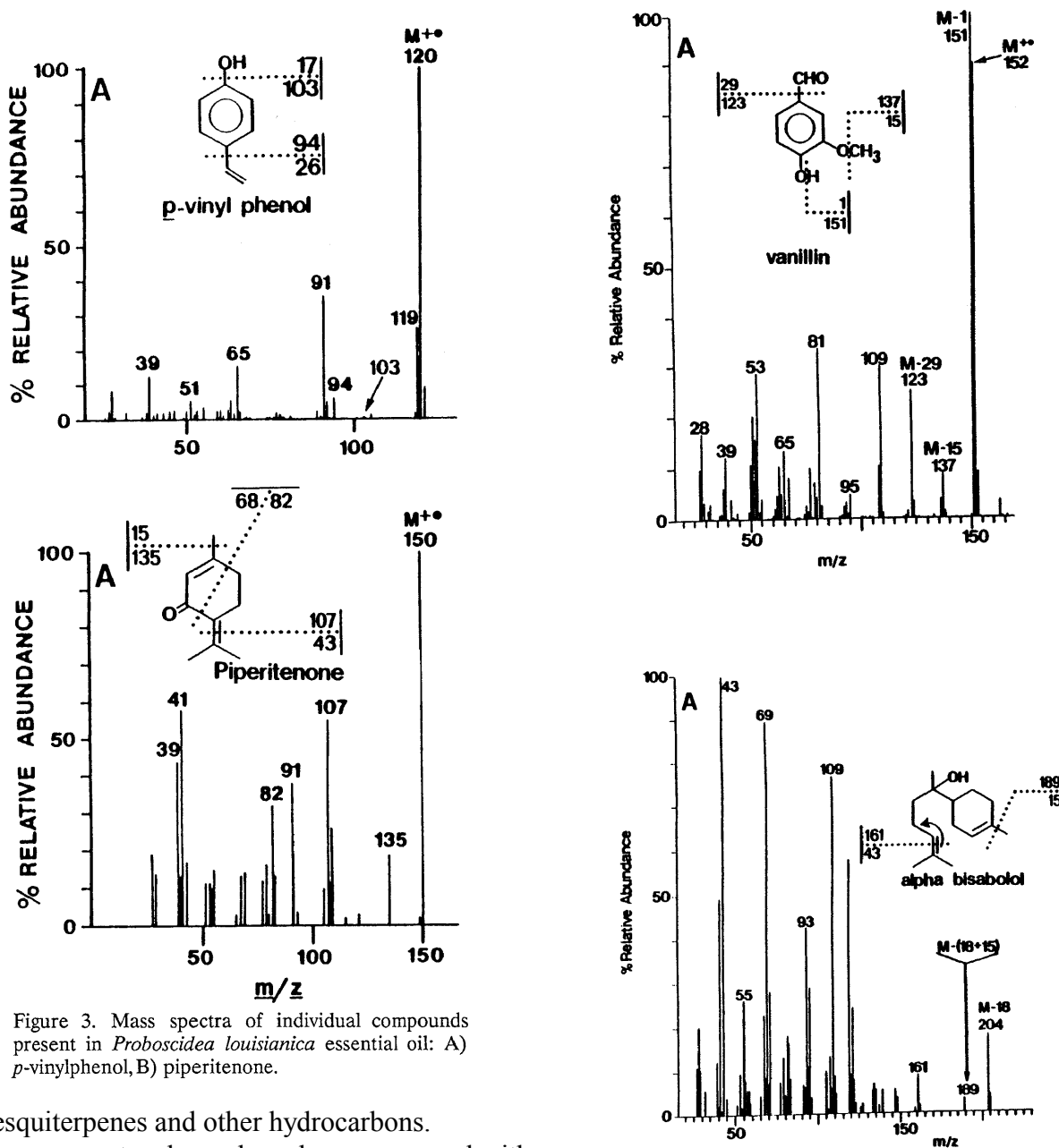


Figure 3. Mass spectra of individual compounds present in *Proboscidea louisianica* essential oil: A) *p*-vinylphenol, B) piperitenone.

Figure 3. Mass spectra of individual compounds present in *Proboscidea louisianica* essential oil: C) vanillin, D) α -bisabolol.

sented sesquiterpenes and other hydrocarbons.

The mass spectra shown have been compared with mass spectral standards from the NBS/EPA/NIH Mass Spectral Data Base (11) except for *p*-vinylphenol, which was run at Stillwater (12), and traxolide, which is available only in the laboratory of International Flavors and Fragrances. International Flavors and Fragrances have their own standards which, in some cases, are more pure than the NBS/EPA/NIH library compounds. Therefore, the mass spectra in some cases do not agree with the NBS/EPA/NIH library data.

The molecular mass of *p*-vinylphenol is 120 (Fig. 3A). The loss of a hydroxyl group gives rise to the ion at $m/z = 103$. An ion at $m/z = 94$ represents the loss of an ethyl group. Piperitenone has a molecular mass of 150 which is also the location of its base peak (Fig. 3B). The ion at $m/z = 135$ represents the loss of a methyl group from the

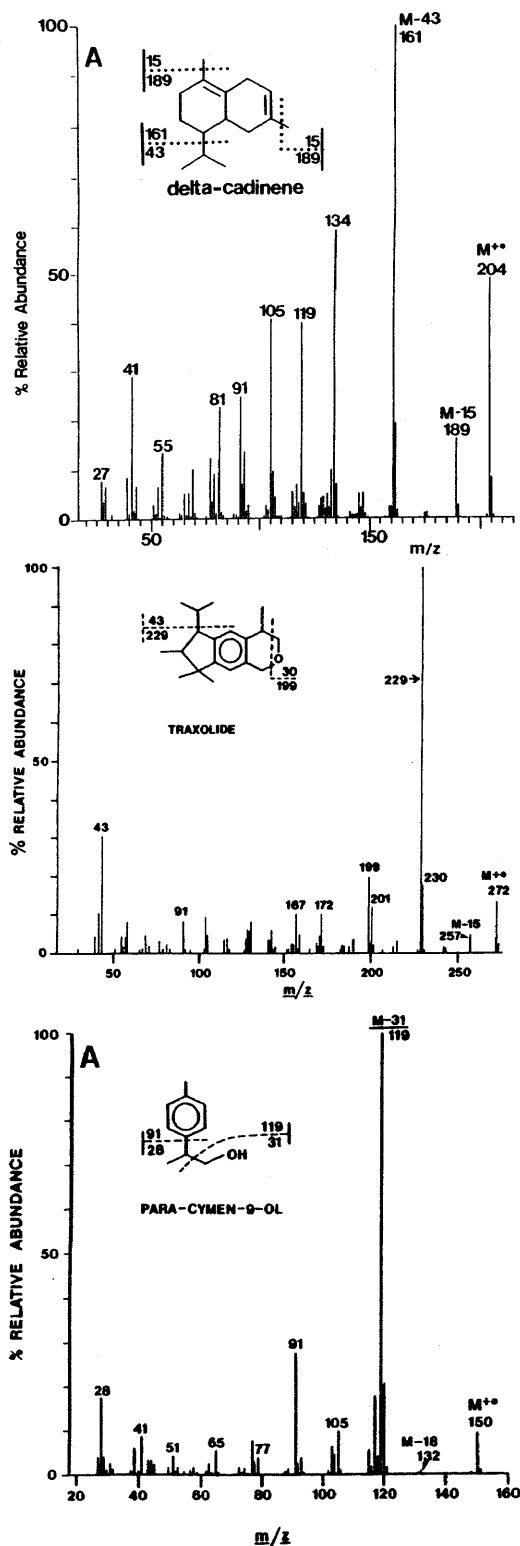


Figure 3. Mass spectra of individual compounds present in *Probooscidea louisianica* essential oil: E) δ -cadinene, F) traxolide, G) *p*-cymen-9-ol.

molecular ion and that at $m/z = 107$ the loss of a isopropyl group.

The molecular mass of vanillin is 152 (Fig. 3C). The loss of a hydrogen results in a prominent $m/z = 151$ base peak. The ion at $m/z = 137$ represents the loss of a methyl group. The loss of a formyl group (CHO) produces an ion at $m/z = 123$. The molecular mass of α -bisabolol is 220 but the loss of H_2O occurs very readily; this is shown in the mass spectrum (Fig. 3D) which contains $m/z=204$, the pseudo molecular ion. The loss of a methyl group results in an ion at $m/z = 189$. The ion at $m/z = 161$ is characteristic of the loss of an isopropyl group.

The molecular mass of δ -cadinene is 204 (Fig. 3E). The loss of either methyl group results in the ion at $m/z = 189$. The loss of the isopropyl group results in a prominent ion at $m/z=161$. The molecular mass of traxolide is 272 (Fig 3F) and the loss of a methyl group gives rise to an ion at $m/z=257$. The $m/z=229$ ion indicates the loss of an isopropyl group. The molecular mass of *p*-cymen-9-ol is 150 (Fig 3G). The ion at $m/z=119$ gives the base peak and represents the loss of the CH_2OH group.

The $m/z = 91$ species represents the tropylium ion and is characteristic of the following spectra of the following compounds: *p*-vinylphenol, piperitenone, α -bisabolol, δ -cadinene, traxolide, and *p*-cymen-9-ol.

DISCUSSION

Devil's-claw essential oil volatilizes from the plant growing in the field and gives a distinct acrid odor to the air around these fields. This release of volatiles is similar to that of many other plants. These volatiles were captured by aspiration/adsorption on activated charcoal and eluted with methanol; the same distinct odor was present in the eluate, but the compound(s) responsible could not be identified due to the very small amount of material obtained.

Waller and Johnson (9) found that when $[G-^{14}C]$ -nepetalactone was administered to *Nepeta cataria* plants, a significant amount of $[G-^{14}C]$ -dihydronepetalactone was bound to plant components and could not be steam distilled out in a normal operation. Analysis by GC and GC/MS showed that treatment of the plant residues with hot 2N HCl liberated four times as much steam-volatile material, which contained radioactive diastereoisomeric

dihydronepetalactones. In the devil's-claw experiments, similar steam distillation from acidified plant material yielded 73% as much as from the stems and leaves, 27% as from the pods, and 147% as from the roots, which was considerably less than found in *N. cataria* essential oil. It is clear that compounds of the bicyclic nepetalactone series are tightly bonded and released only by steam of acidified plant material, whereas similar treatment of devil's-claw plant material yielded totally different compounds with vanillin being the only compound found in both normal and acid-released essential oil.

A common volatile sesquiterpene, δ -cadinene, is a moderately abundant constituent of devil's-claw essential oil and is also found in *Siparuna guianensis* leaves (13), in wheat leaves (14), and in the essential oils of *Sideritis* spp. (15), clove (*Eugenia caryophyllus*) (16), and *Rhus typhina* (17). Monoterpene aldehydes, alcohols, and hydrocarbons make up much of the floral fragrance of *Platanthera stricta* (18). Many terpenoid compounds attract insects (14, 19) e.g., δ -cadinene.

Piperitenone is present in relatively small amounts in devil's-claw essential oil. This ketone is present in minute quantities in *Sideritis* spp. essential oil (15). α -Bisabolol, a hydroxy derivative of bisabolene, is present in moderate amounts in devil's-claw essential oil. Bisabolene is not very common in essential oils but is found in small amounts in the constituents of the rhizome of calamus (*Acorus calamus*) (20).

Devil's-claw essential oil contains small amounts of 6-methyl-5-hepten-2-one; clove essential oil contains this compound (16). The volatile constituents of *Amaranthus palmeri* seedheads were rich in 2-heptenone; vapors of these compounds at a concentration of 1 ppm in air strongly inhibited the germination of onion (*Allium cepa*) and carrot (*Daucus carota*) and almost completely suppressed that of tomato (*Lycopersicon esculentum*) (21).

Phenolic compounds such as vanillin are commonly found in the soil. They are released as root exudates or from decomposing plant litter (22). Vanillin is ubiquitous in soil since it is a degradation product of lignin (23). Phenolic compounds can act as plant growth inhibitors when present in the soil (24). Vanillin is a major constituent of devil's-claw essential oil and is most likely released in large amounts by decomposing plants late in the summer when the plants senesce and die. It probably is not released in a significant quantity by wind blowing the devil's-claw plant; however, this remains to be proven.

The volatile constituents of kumquat (*Fortunella margarita*) essential oil contain *p*-cymene (25). Kumquat essential oil is similar to devil's-claw essential oil in that it contains many mono- and sesquiterpenes and other hydrocarbons. The essential oil of *Sideritis* spp. contains *p*-cymene and *p*-cymen-8-ol (14) and the latter compound is very similar to the *p*-cymen-9-ol found in the essential oil released from devil's claw by the HCl treatment.

Riffle et al. (8) reported that cotton exhibited necrotic and senescent tissue on leaf margins when growing in close association with devil's-claw. It is not known whether the response of the cotton leaf is due to volatile substances from devil's-claw blown through the air into the cotton plant or due to actual contact by the cotton leaf with the devil's claw glandular hairs. Whichever the case, necrosis of the cotton leaves is probably an allelochemical effect of the devil's-claw plant.

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