

Non-terpenoid essential oils from *Bursera chemapodicta*

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ABSTRACT: The essential oils of the neotropical deciduous tree *Bursera chemapodicta* are short-chain alkanes and alkanolic derivatives, which contrast to the terpenoid resins of other characterized *Bursera* species. The resin composition of *B. chemapodicta* differs from leaf to twig, indicating a tissue specificity for non-terpenoid essential oil synthesis. Field-collected leaves contained an average of 4.38% heptane and 5.32% total semi-volatiles. This is a major switch in resin chemistry from terpenoid to linear alkanes within the genus *Bursera*. Copyright © 2006 John Wiley & Sons, Ltd.

KEY WORDS: *Bursera chemapodicta*; essential oils chemistry; alkane oleoresins; Burseraceae

Introduction

The family Burseraceae of tropical and temperate deciduous trees comprises approximately 600 species in 20 genera, many of which contain resins that bleed to produce an aromatic gum.^{1,2} Old World plants in this family are the source of frankincense (*Boswellia*) and myrrh (*Commiphora*). The New World genus *Bursera* contains about 100 species distributed from the south-western USA to Peru.^{3,4} Many Burseraceae produce copious resin from arborescent canals, providing a defence against herbivory.^{5–8} These resins have a long history of medicinal and ceremonial use in Mesoamerica.⁹

As with many other Burseraceae, the primary chemical constituents of *Bursera*'s resin are terpenoid essential oils.^{10,11} Many terpenes are known to be deterrent and toxic to insects, fungi and bacterial pathogens.^{12,13} The genus *Bursera* is the main host of *Blepharida* (Chrysomelidae: Alticinae) beetles, consisting of about 45 species, many of which are monophagous.^{14,15} Resins in *Bursera* decrease *Blepharida* survival and growth rate;^{6,7} however, some *Blepharida* species depend on *Bursera*'s secondary chemistry for their own defence.¹¹ *B. chemapodicta* was described as a species distinct from *B. schlehtendalii*, substantially on the basis of differing essential oil composition. We wished to characterize the essential oil composition of *B. chemapodicta* and determine whether the chemistry may account for a difference in *Blepharida* herbivory.

Experimental

Plant Sources

B. chemapodicta plants were collected in Xochipala, Cañon del Zopilote, Guerrero, Mexico, and subsequently grown in Tucson, Arizona. Field-collected leaves from three individual *B. chemapodicta* plants were collected on the north bank of the Balsas River, Guerrero, Mexico, 17°57.071'N, 99°21.552'W, on 15 July 2003.

Extraction of Plant Constituents

Organosoluble constituents of leaves and twigs were extracted with 20× (wt/vol) dichloromethane for 24 h. Volatile organic compounds emitted from twigs and leaves of *B. chemapodicta* were concentrated by absorption onto a 100 µm polydimethylsiloxane coated solid-phase microextraction (SPME) fibre (Supelco, Bellefonte, PA) from approximately 0.1 g each of twig or leaf placed into a 7 ml vial and sealed with Teflon tape. The SPME fibre was exposed to the headspace over plant tissue for 5 min at 50 °C.

Analysis of Essential Oils

Gas chromatography–mass spectrometry of *B. chemapodicta* resin was performed on a Hewlett-Packard 5890 gas chromatograph with a 5970B mass selective detector at 70 eV, *m/z* 40–600 full scan on a DB-5 (J&W Scientific, Folsom, CA) 25 m × 0.32 mm i.d., 0.52 µm column, using helium as the carrier gas at a linear velocity of 28 cm/s. The splitless injector temperature was 200 °C, the detector temperature 280 °C, and the oven programmed from 40 °C to 230 °C at 10 °C/min. The plant essential oils, extracted with dichloromethane, were identified by comparison with synthetic or natural standards, matching gas chromatographic retention times and mass spectra. Quantification of volatile essential oils from field-collected

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plants was by gas chromatography with flame ionization detection, using 1-octanol as an internal standard.

Sources of Reference Compounds

2-Heptanol was purchased from Aldrich Chemical Co., 1-octanol from Matheson Co., caryophyllene from Givaudan, isopropyl alcohol from Baker and eugenol from Sigma. Nonane, undecane, 1-hexanol, heptanol, 2-nonanol, methyl isobutyl ketone, and 4-methylhexan-3-one were gifts from Justin Schmidt of the Carl Hayden Honeybee Laboratory, USDA, Tucson, AZ, USA.

Synthesis of Reference Compounds

Acetate esters were synthesized from the appropriate alcohols by sulphuric acid-catalysed acetylation in excess acetic acid, giving from the corresponding alcohols 1-methylhexyl acetate, hexyl acetate, octyl acetate, and heptyl acetate. 2-Nonanone was synthesized by dichromate oxidation of 2-nonanol. 4-Methyl-2-pentyl acetate was synthesized by acetylation of isobutylmethylcarbinol, produced by the lithium aluminium hydride reduction of methylisobutylketone. 4-Methyl-3-hexanol acetate was synthesized by acetylation of 4-methylhexan-3-ol, produced by lithium aluminium hydride reduction of 4-methylhexan-3-one. 4-Cymene was synthesized by sulphuric acid-catalysed alkylation of toluene with isopropyl alcohol.

Results and Discussion

Bursera chemapodicta is an endemic species from the Cañon del Zopilote region of the state of Guerrero, Mexico. It was originally described as a species distinct

from its close relative *B. schlehtendalli* Engl., based on the gas chromatographic pattern of the resin¹⁶ and by its conspicuous pubescence.

During our analysis of resin extracted from collected *B. chemapodicta* we found the essential oils were not the expected terpenes but primarily non-terpenoid short chain aliphatic alkanes, acetates, alcohols and ketones. The major extracted volatile essential oil constituents of leaves and twigs of *B. chemapodicta* are heptane and the oxygenated 7-carbon compounds 2-heptanol, 1-methylhexyl acetate and octyl acetate.

Monoterpenes are conspicuously absent from both leaf and twig *B. chemapodicta* extracts; however, the sesquiterpene caryophyllene and small amounts of other spectrally indicated but unidentified sesquiterpenes are present. Solid-phase microextraction of *B. chemapodicta* reveals heptane, 2-heptanol and 1-methylhexyl acetate as the major compounds released from the leaves, and heptane, 4-methyl-3-hexanol acetate and 1-methylhexyl acetate from the twigs (Table 1). The pattern of solvent-extracted volatile and semi-volatile constituents differs from leaves to twigs, with the twigs having a higher percentage of heptane and lacking in 2-heptanol, a major compound in the leaves.

To quantify the amounts of non-terpenoid resins in naturally grown *B. chemapodicta*, we collected leaf samples from three individual plants in the summer of 2003. The dichloromethane extracted essential oils averaged 5.32% of the leaf dry weight, with 4.38% of leaf dry weight being heptane. Live plants most likely contain higher amounts of resin than these numbers indicate, since a considerable amount of pressurized resin is lost by being squirted out as the leaf is severed from the stem during collection.

Table 1. Percentage composition of identified extracted and volatile components of *Bursera chemapodicta*

Constituent	Leaf extract (%)*		Twig extract (%)*	
	SPME	Solvent	SPME	Solvent
Heptane	22.5	68.4	19.4	74.0
2-Heptanone	3.2			
3-Heptanol	2.6			
2-Heptanol	26.4	3.1		
Nonane			6.0	2.4
4-Methyl-2-pentyl acetate			1.5	0.3
Acetic acid, hexyl ester			1.6	
4-Methyl-3-hexanol acetate	5.4	2.2	15	3.4
1-Methylhexyl acetate	40.0	10.0	51.0	17.7
2-Nonanone			0.8	
Acetic acid, heptyl ester		2.1	1.9	0.5
Octyl acetate		8.4	0.9	0.7
1-Nonene			0.9	0.5
Caryophyllene		3.9		

Total percentages <100 are due to the presence of minor unidentified compounds.

* SPME, solid phase microextraction; the solvent is dichloromethane.

Biosynthesis of large amounts of alkanes and alkanolic acid derivatives, such as heptane, 2-heptanol and 1-methylhexyl acetate, in the resin of *B. chemapodicta* is evidence of a fundamental metabolic shift in oleoresin production away from monoterpenes in sister species, although few other highly resinous vascular plants are known to have a similar alternative resin chemistry. Within the Old World Burseraceae the resin of *Commiphora rostrata* Engl. consists primarily of the short-chain ketones 2-decanone, methylnonyl ketone, 2-dodecanone and hexadecanal.⁵ In most conifers the oleoresins are terpenoid, an exception being the Jeffrey pine (*Pinus jeffreyi* Grev. & Balf.), in which heptane is the major oleoresin component.^{17–19} Jeffrey pine is closely related to the common terpenoid resin-producing *Pinus ponderosa* Dougl.

Based on current understanding of the biosynthesis of vascular plant resins, the production of terpenoid and non-isoprenoid compounds diverges early in the pathway of anabolic plant secondary compound synthesis. Consistent with known biosynthetic routes of hydrocarbon synthesis in the peanut (*Arachis hypogaea*) and the Jeffrey pine (*P. jeffreyi*), the short-chain alkane resin of *B. chemapodicta* may originate from the polymerization of acetate via fatty acid synthase.²⁰ That the released volatile and extracted resin components differ between leaves and twigs in *B. chemapodicta* indicates a tissue-specific localization or differentiation in hydrocarbon resin synthesis similar to the finding in the Jeffrey pine.^{19,21} Caryophyllene, a sesquiterpene, is found in the high alkane resin-producing *B. chemapodicta*, demonstrating that alkane resin species retain at least their sesquiterpenoid synthetic capabilities.

In Jeffrey pine, the high level of alkanes in the resin is associated with resistance to the polyphagous bark beetle *Dendroctonus ponderosae* Hopk. (Coleoptera: Scolytidae) and correlated with the host specificity of the monophagous bark beetle *D. jeffreyi* Hopk.^{22,23} McDowell *et al.*⁵ concluded that the oxygenated alkanolic acid derivatives in the resin of *C. rostrata* were responsible for defence against herbivores and fungal pathogens. *B.*

chemapodicta has largely replaced the terpene resins with biosynthetically unrelated short-chain alkanes, and oxygenated alkanolic acid derivatives having physical properties of polarity, vapour pressure and viscosity similar to the common monoterpenes. This new oleoresin composition may present a significant chemical barrier to potential herbivores and pathogens.

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