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RAPID COMMUNICATION

The Pattern of Straight Chain Hydrocarbons Released by *Yucca* Flowers (Asparagaceae)

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Abstract

The hydrocarbon pattern in the floral scent of *Yucca* species was found to comprise a group of unbranched, mid-chain alkanes, alkenes, and an alkadiene. In *Y. reverchonii,* highly dominant (*Z*)-8-heptadecene is accompanied by (6*Z*,9*Z*)-6,9-heptadecadiene and heptadecane as minor components and by traces of other saturated and unsaturated hydrocarbons with similar chain length. Some of these volatiles proved to be perceived by the antennae of *Tegeticula cassandra* (pollinating seed-eater of *Yucca*) and *Prodoxus decipiens* (herbivore on *Yucca*). The possible biosynthesis of the compounds is discussed.

Keywords *Yucca* · Scent · Hydrocarbons · (*Z*)-8-Heptadecene · Biosynthesis

Introduction

The intimate relationship between *Yucca* (Asparagaceae) and its moth pollinators of the genera *Tegeticula* and *Parategeticula* (Prodoxidae) has received major attention by biologists since its discovery by Engelmann almost 150 years ago (Engelmann 1872; Pellmyr 2003). Although this is a well-studied example of mutualism and coevolution, the chemical structures of sensory signals mediating attraction of the pollinators to *Yucca* are largely unknown. Yuccas are typically pollinated during night, which is the time when their white flowers are open and the fragrance is most pronounced. Indeed, floral scent has been suggested as a key sensory cue for the moths during host location and discrimination (Svensson et al. 2011). According to present knowledge, *Yucca* volatiles are typically comprised of terpenoids (isoprenoids) and straight chain hydrocarbons (acetogenins), however, only little is known about the chemical structures of these compounds (Svensson et al. 2005; Wang and Kameoka 1980).

The qualitative patterns of the two classes of compounds look more or less the same in all *Yucca* species investigated so far, however, there are differences in relative proportions. Some species such as *Y. reverchonii* almost exclusively release straight chain alkanes and alkenes, the structure elucidation of which is the subject of the present paper. Our investigations may help reveal the chemistry and biological significance of volatile signals released by *Yucca* species to attract their highly specialized pollinators.

Material and Methods

Collection of Floral Scent

Yucca reverchonii floral headspace was collected in an outdoor common research garden at Syracuse University, Onondaga Co., NY, using plants transplanted from a natural population in Sonora, Sutton Co., TX (N 30° 43.518' N, 100° 10.635' W). Collections were performed on 8 July, 2013, and 9 July, 2014 (total of 6 floral samples plus ambient controls), by placing a "turkey sized" (482 × 596 mm) nylon resin oven bag (Reynolds Consumer Products, Inc. Lake Forest, IL, USA) over a blooming inflorescence, secured around the scape with a plastic tie. Volatiles were trapped using a glass

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cartridge (7 mm i.d.) filled with 100 mg of Super Q adsorbent (Alltech Associates, State College, PA, USA), which was inserted into the bag and connected to a 9 V battery operated PAS-500 personal air sampler pump (Supelco, Bellefonte, PA, USA), with a flow rate of 200 ml/min. Scent collection was performed from 20:00 h to midnight. Adsorbed volatiles were eluted from the Super Q trap with 3 ml of hexane (GC2; Burdick and Jackson, Inc. Muskegon, MI, USA) and stored at −18 °C until analysis. Empty bags were used as ambient controls to check for possible contaminants emitted from the bag. Prior to analysis by gas chromatography coupled with mass spectrometry (GC-MS), samples were concentrated to 75 μl under a gentle flow of nitrogen.

Chemical Analysis and Synthesis of Floral Volatiles

Floral headspace was analyzed by using a quadrupole mass spectrometer 5975C inert XL MSD (run at 70 eV), which was linked to a 7890A gas chromatograph (both Agilent Technologies, Santa Clara, CA, USA). Separation of volatiles was achieved with a HP-5 ms fused silica capillary column, 50 m × 0.25 mm i.d. and 0.25 μm film thickness (J&W Scientific, Folsom, CA, USA). Helium served as the carrier gas at a velocity of 25 cm/min and an injector temperature of 250 °C. After splitless injection (30 s) the oven temperature was kept at 50 °C for 3 min and then programmed to 80 °C at a rate of 3 °C/min, followed by an increase of 5 °C/min to 150 °C and finally of 7.5 °C/min to 300 °C. Scent data were analyzed using the software ChemStation E.02.02.1431.

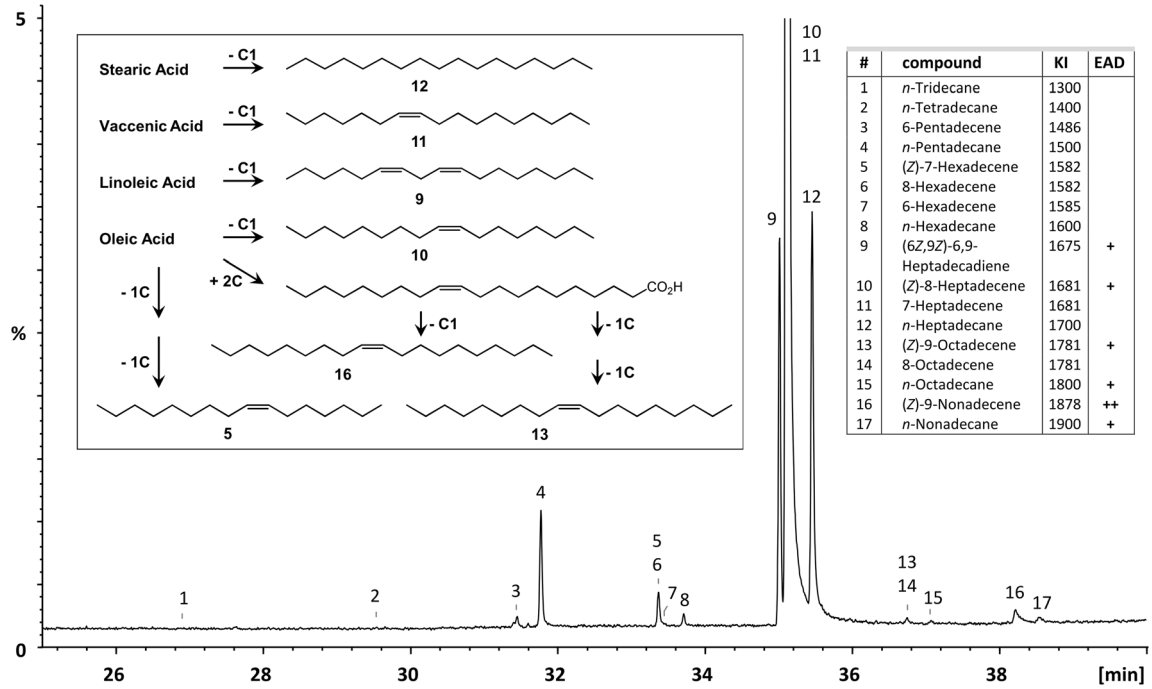
Reagents and solvents used for the synthesis of *Yucca*-volatiles were purchased from Sigma-Aldrich (Merck), Darmstadt, Germany. Positions of double bonds in alkenes were determined by methylthiolation (Francis and Veland 1981; Vincenti et al. 1987). *E/Z*-Mixtures of alkenes were synthesized by Wittig reaction of an appropriate aldehyde and a corresponding triphenylphosphine-alkylene, using hexamethyldisilazane as the base ("salt-free" method) (Reitz et al. 1986). Commercial linoleic acid served as starting material for the synthesis of (*Z*6,*Z*9)-6,9-heptadecadiene (van der Klis et al. 2011), however, for the oxygenation reaction we used ammonium peroxodisulfate instead of the sodium salt.

Electrophysiology

To check whether yucca moths can detect the hydrocarbons produced by *Yucca* hosts, GC with simultaneous flame ionisation detection (FID) and electroantennographic detection (EAD) was applied. For these analyses a Shimadzu GC-17A gas chromatograph equipped with a DB-5 column (30 m × 0.32 mm i.d., and 1 mm film thickness, (Agilent Technologies, Santa Clara, CA, USA) was used. Hydrogen served as carrier gas at a velocity of 43 cm/s, and the injector temperature was 270 °C. The oven temperature was maintained at 50 °C for 3 min after injection and then increased at 10 °C/min to 275 °C. A glass Y connector (J&W Scientific, Folsom, CA, USA) at the end of the column allowed a 1:1 division of the GC effluent to the FID and to the antennal preparation. Because no yucca moths associated with *Y. reverchonii* were available, we instead used *Tegeticula cassandra* and *Prodoxus decipiens* in the electrophysiological screening. These moths feed on *Y. filamentosa*, a species producing a similar set of hydrocarbons as *Y. reverchonii*. An antenna was cut at the base and at the tip, mounted between two glass electrodes filled with saline solution, and placed 1 cm from the glass tube of the GC outlet. The air stream through the glass tube was charcoal-filtrated, humidified, and presented at a flow rate of approximately 200 ml/min. The GC effluent to the antennal preparation passed through a heated transfer line (Syntech, Kirchzarten, Germany) set at 275 °C. Recordings were analysed using the Shimadzu Class-VP version 7.2.1 software. Electrophysiologically active compounds were assigned using a second Shimadzu GC-17A gas chromatograph linked to a Shimadzu QP5000 mass spectrometer run in EI-mode at 70 eV. The same column type and temperature conditions were used as for the GC-EAD analyses, except for the initial oven temperature, which was kept at 50 °C for 2 min. Helium was used as carrier gas at a velocity of 43 cm/s. Compounds in samples were identified by comparing mass spectra and retention times with those of authentic references.

Results

The bouquet of hydrocarbons released by flowers of *Y. reverchonii* is largely comprised of straight chain alkanes, alkenes, and an alkadiene with 11–19 carbon atoms and a maximum at C-17 (Fig. 1). These compounds were consistent across 6 floral samples and were absent in ambient control samples. Structures of *n*-alkanes were assigned on the basis of known mass spectra and co-injection. According to derivatization reactions, the alkenes show double bonds in mid-chain positions and are highly dominated by (*Z*)-8-heptadecene. For some of the natural products *Z*-configuration could be assigned by comparison of retention times and co-injection with synthetic reference samples. Generally, the applied "salt-free" variant of the Wittig reaction produces almost exclusively *Z*-alkenes (Reitz et al. 1986). Under the analytical conditions used in the present investigation, *Z*-alkenes and the later eluting *E*-isomers were well separated with an α-value of Rt*E*:Rt*Z* = 1002 (Rt 9*E*-C19-ene = 38,32 min, KI = 1883 and Rt 9*Z*-C19-ene = 38,24 min, KI = 1878). This is equivalent to a difference in retention times of ca 5 s, causing baseline separation. Therefore, stereochemical assignments of the more abundant *Z*-alkenes were simple and unambiguous. For some trace components the configuration could not be



#	compound	KI	EAD
1	*n*-Tridecane	1300	
2	*n*-Tetradecane	1400	
3	6-Pentadecene	1486	
4	*n*-Pentadecane	1500	
5	(*Z*)-7-Hexadecene	1582	
6	8-Hexadecene	1582	
7	6-Hexadecene	1585	
8	*n*-Hexadecane	1600	
9	(6*Z*,9*Z*)-6,9-Heptadecadiene	1675	+
10	(*Z*)-8-Heptadecene	1681	+
11	7-Heptadecene	1681	
12	*n*-Heptadecane	1700	
13	(*Z*)-9-Octadecene	1781	+
14	8-Octadecene	1781	
15	*n*-Octadecane	1800	+
16	(*Z*)-9-Nonadecene	1878	++
17	*n*-Nonadecane	1900	+

Fig. 1 *Yucca reverchonii*: gas chromatogram and list of compounds, corresponding Kováts-indices (KI, according to Kováts 1965), and data concerning EAD-activities; for conditions see text. The insert illustrates the possible biosynthesis of straight chain *Yucca* hydrocarbons

determined, due to very small amounts and partial co-elution with major alkene isomers. Interestingly, some alkenes were found to be mixtures of positional isomers: hexadecenes are represented by the (*Z*)-7-isomer (major) and trace amounts of the 6- and 8-isomers. Synthetic (Z6,Z9)-6,9-heptadecadiene co-eluted with the natural diene, which, along with *n*-heptadecane, was the second most abundant compound in the bouquet (Fig. 1).

Discussion

Here we provide the first detailed information about the chemical structures of the straight chain hydrocarbons released by a *Yucca* species under natural conditions. The qualitative pattern of hydrocarbons found in *Y. reverchonii* is largely the same as in other species in the genus (Svensson et al. 2005, 2011; Wang and Kameoka 1980), however, there are distinct quantitative differences. The most abundant component is (Z)-8-heptadecene, which we also found in other *Yucca* species (Tröger, unpublished). Earlier, this compound had been erroneously described to be 1-heptadecene (Svensson et al. 2005, 2011), but had been correctly identified from the essential oil of *Y. gloriosa* and two other *Yucca* species (Wang and Kameoka 1980). Our study confirms that this compound is an actual *Yucca* floral volatile, rather than an artefact of steam distillation.

The unusual hydrocarbon-dominated bouquet of *Y. reverchonii* cannot be an artefact of transplanting, because transplanted *Y. glauca* plants in the same common garden produced the same blends of terpenoids and hydrocarbons with unknown structures (analysed by GC-MS, data not shown) previously documented from natural populations of that species (Svensson et al. 2011).

The biosynthesis of *Yucca* hydrocarbons has not been investigated so far, however, some plausible suggestions can be made on the basis of present knowledge on the formation of such compounds. Realistic precursors of odd numbered *n*-alkanes (1, 4, 12, 17 in Fig. 1) are saturated fatty acids, which lose the carboxyl group by decarboxylation (Jurenka 2004 and literature cited therein). The scenario is explained in Fig. 1, insert. Stearic acid will yield heptadecane (12). Consequently, (Z)-9-octadecenoic acid (oleic acid) will be the precursor of (*Z*)-8-heptadecene (10), while (9*Z*,12*Z*)-9,12-octadecadienoic acid (linoleic acid) will be transformed to (6*Z*,9*Z*)-6,9-heptadecadiene (9). Similarly, 11-octadecenoic acid (vaccenic acid) may serve as the precursor of 7-heptadecene (11), which coelutes with (*Z*)-8-heptadecene (10). Chain elongation of oleic acid with malonate leads to (*Z*)-11-eicosenoic acid (gondoic acid) which will form (*Z*)-9-nonadecene (17) upon decarboxylation. The biosynthesis of even numbered hydrocarbons is more complex: in the case of (*Z*)-9-octadecene (13), gondoic acid is chain shortened in two consecutive decarboxylation steps (Goller et al. 2007). Similarly, (*Z*)-7-hexadecene (5) would be formed from oleic acid. Possible biosynthesis of some alkenes with unknown stereochemistry (6-pentadecene, 8- and 6-hexadecene, and 8-octadecene, see Fig. 1) will not be discussed here.

Electrophysiological investigations have revealed that some of the identified hydrocarbons, particularly (*Z*)-9-nonadecene (see Fig. 1), elicit antennal responses in several species of *Tegeticula* moths, the specialized seed feeding pollinators of *Yucca* and also in species of the genus *Prodoxus*, which are specialized herbivores of *Yucca*, but feed on plant parts other than seeds and do not contribute to pollination (Svensson, Raguso, unpublished). Investigations on the biological significance of the hydrocarbons identified here are under way; whether they are behaviorally active to pollinator moths and are useful in chemotaxonomic approaches awaits further investigations.

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