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High-Density Biosynthetic Fuels: The Intersection of Heterogeneous Catalysis and Metabolic Engineering

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Abstract

Biosynthetic valencene, premnaspirodiene, and natural caryophyllene were hydrogenated and evaluated as high performance fuels. The parent sesquiterpenes were then isomerized to complex mixtures of hydrocarbons with the heterogeneous acid catalyst Nafion SAC-13. High density fuels with net heats of combustion ranging from 133-141,000 btu/gal, or up to 13% higher than commercial jet fuel could be generated by this approach. The products of caryophyllene isomerization were primarily tricyclic hydrocarbons which after hydrogenation increased the fuel density by 6%. The isomerization of valencene and premnaspirodiene also generated a variety of sesquiterpenes, but in both cases the dominant product was δ-selinene. Ab initio calculations were conducted to determine the total electronic energies for the reactants and products. In all cases the results were in excellent agreement with the experimental distribution of isomers. The cetane numbers for the sesquiterpane fuels ranged from 20-32 and were highly dependent on the isomer distribution. Specific distillation cuts may have the potential to act as high density diesel fuels, while use of these hydrocarbons as additives to jet fuel has the potential to increase the range and/or time of flight of aircraft. In addition to the ability to generate high performance renewable fuels, the powerful combination of metabolic engineering and heterogeneous catalysis allows for the preparation of a variety of sesquiterpenes with potential for pharmaceutical, flavor, and fragrance applications.

Introduction

Over the last several years considerable effort has been expended on the development of full-performance renewable jet and diesel fuels. Although significant progress has been made, the search for methods that balance carbon efficiency with the production of optimized biofuels continues.[1] This search has resulted in the evolution of several generations of biofuels.[2] The first generation is widely characterized as being composed of fuels such as corn-based ethanol and fatty acid methyl esters (FAME) derived from plant oils. In the case of ethanol, although a serviceable gasoline-range fuel, it has a low net heat of combustion (~70% of gasoline), is completely miscible with water, and is much more corrosive than conventional petroleum based fuels.[3] FAME, although a good surrogate for diesel fuel,[4] has a relatively high melting point, is predisposed to biofouling,[5] is more corrosive than petroleum-based diesel,[6] and sustainable production is limited to the availability of oil crops.

The limitations of first generation renewable fuels have led to extensive research aimed at production of second generation, full perfomance jet and diesel fuels from non-food oils and lignocellulosic sources.[7] Within this class of fuels a variety of renewable jet and diesel fuels have been developed that can be broadly classified as synthetic parafinnic kerosene (SPK).[8] SPK can be produced via Fischer-Tropsch catalysis, but in the U.S. is more commonly derived from plant or algal oils. In the case of plant based oils, the production of these fuels is again limited by the availability of oil crops. Preferred feedstocks for these fuels include non-food oils such as camelina oil,[9] however, the modest yield of oil per acre, and thus enormous land and water requirements for plant oil-based fuels represent significant challenges to their widespread use. Microalgae-derived oils hold the promise of much greater productivity,[10] but despite initial results, the production of algae oil faces a number of challenges ranging from harvesting the oil to maintaining high rates of production in open ponds. In terms of performance, SPK fuels, which are composed of linear and branched alkanes, are excellent for jet and diesel propulsion, although they have moderate densities due to the lack of aromatics or cyclic hydrocarbons (napthenes). This lower density and lack of aromatics has required blending of SPK fuels with conventional jet fuel to meet specifications.[11]

An alternative approach to the generation of SPK fuels, broadly classified as alcohol-to-jet fuels (ATJ), is currently emerging as a viable technology. These fuels are synthesized from renewable alcohols that can be produced from lignocellulosic biomass. Recent studies have concluded that sustainable waste biomass can potentially be used as a feedstock to replace up to 30% of U.S. transportation fuels.[12] Fuel production on this scale could result in a significant decrease of carbon emissions in the U.S. In general, these fuels are produced by dehydrating the alcohols to olefins and then selectively oligomerizing the olefins to generate fuels.[2,13-17] Alternatively, fuels can be constructed through Guerbet chemistry in which an alcohol is oxidized to an aldehyde, coupled to additional aldehydes through aldol condensation reactions, and then reduced to a hydrocarbon.[18] Regardless of the process, these fuels have similar properties to other SPK fuels and represent a bottom-up approach in which organisms generate small molecules that are then deoxygenated and combined to produce longer chain fuel molecules.

In contrast to the bottom-up approach represented by Fischer-Tropsch and ATJ fuels, a number of research groups have developed biosynthetic approaches in which microorganisms directly generate larger hydrocarbons required for jet and diesel fuel.[19-21] After direct fermentation to generate a fuel-like molecule, these hydrocarbons are then converted to stable, high-performance fuels through straightforward chemical processes including hydrogenation, isomerization, and distillation. This approach has the potential to reduce capital costs by removing much of the chemical processing required for bottom-up methods, while utilizing either CO_2 or biomass-derived sugars as the carbon feedstock to produce fuels. Remarkable progress has been made in the direct production of renewable fuels and oils by organisms including cyanobacteria,[22] bacteria, algae, and yeast. Much of this work has focused on long chain linear alkanes[23] and alkenes.[24]

One of the most promising approaches to high-performance biosynthetic fuels is to utilize the tools of metabolic engineering to overproduce specific molecules with structures of interest. Terpenoid structures are an obvious choice based on the vast number of naturally occurring terpenoids (~50,000)[25,26] and the structural diversity of these molecules including branched chain, cyclic, and multicyclic hydrocarbons. In particular, monoterpenes (C10) and sesquiterpenes (C15) are of interest for both jet and diesel fuels. Farnesane (Figure 1) has already been commercialized as a renewable diesel fuel,[27] while hydrogenated cyclic terpenes derived from limonene, pinenes, and sabinene are being considered as components of jet fuel.[28] Although farnesane can be used as a renewable diesel

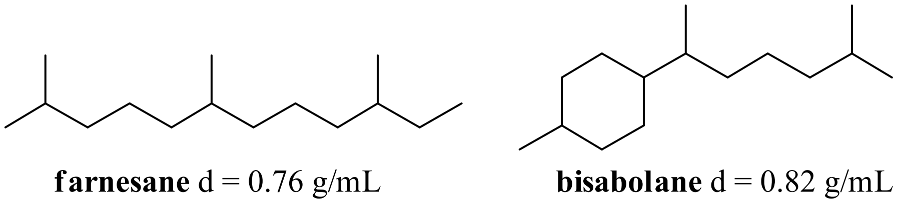


Figure 1. Structures of sesquiterpene fuels

fuel and component of jet fuel, it suffers from the same moderate density as the SPK fuels. A logical way to improve the density of renewable fuels is to incorporate cyclic molecules that have acceptable combustion properties. Although this could be accomplished through a formulation or blending approach, a more elegant route lies in the directed biosynthesis of cyclic sesquiterpenes. A recent paper has demonstrated the biosynthesis of bisabolene (Figure 1) which combines the combustion properties of a linear alkane with the increased density of a cyclic hydrocarbon.[29] The hydrogenated version has a density of 0.82 g/mL which is similar to Jet-A. This work represents an impressive first step in the development of full-performance biosynthetic fuels from biomass. However, to develop renewable fuels that have the potential to outperform conventional petroleum fuels in regard to density and net heat of combustion, the current work explores the synthesis and fuel properties of both pure hydrogenated multicyclic sesquiterpenes and complex mixtures of isomerized sesquiterpenes.

Experimental

General. Valencene and premnaspirodiene were biosynthesized from glucose and provided by Allylix Inc. β-caryophyllene (FCC grade), 10 % Pd/C, PtO_2, Nafion SAC-13, and MMT-K10 were all purchased from Aldrich and used without purification. The β-caryophyllene was 92% pure as measured by GC and was used as received. NMR spectra were collected on a Bruker Avance II 300 MHz NMR spectrometer. Samples were prepared in $CDCl_3$ and spectra were referenced to the solvent peaks (δ 7.26 and 77.16 ppm for ^{1}H and ^{13}C spectra, respectively). Net heat of combustion (NHOC) measurements were conducted at the Southwest Research Institute (SWRI) using ASTM D240N. Viscosity measurements were either conducted at SWRI using ASTM D445, or in our laboratories as previously described.[30] Ignition quality testing (IQT) was conducted at SWRI using ASTM D6890.

Isomerization of β-caryophyllene with Nafion SAC-13. β-caryophyllene was isomerized by vigorously stirring heterogeneous mixtures of the reactant and catalyst (Nafion SAC-13) under nitrogen at 100 (+/-5) °C for 24 h. The catalyst loading was either 1 g cat/100 mL β-caryophyllene (high catalyst loading) or 0.4 g cat/100 mL β-caryophyllene (low catalyst loading). Isomer mixtures were obtained by simple decantation of the catalyst mixture.

Isomerization of β-caryophyllene with MMT-K10. β-caryophyllene (100 g) was added to a flask charged with MMT-K10 (2 g) and the mixture was slowly heated under nitrogen to 100 ˚C. When the reaction temperature reached 80 ˚C the reaction became very exothermic and the temperature rapidly increased to 100 °C. Within 15 min the reaction mixture became viscous and dark brown. Samples were taken from the reaction mixture at 1, 2, and 3 h for GC analysis. The sample taken at 1 h showed about 40% conversion to dimer. Subsequent samples showed no significant change in the distribution of products.

Isomerization of valencene and premnaspirodiene. Nafion SAC-13 (0.1 g) was added to a flask containing sesquiterpene (5 g) and the mixture was heated under nitrogen to 100 °C. Samples were withdrawn via syringe for NMR, GC/FID, and GC/MS analysis after 4 and 24 h.

General procedure for hydrogenation and purification of sesquiterpenes. Hydrogenation was conducted in a Parr shaker without the addition of solvent at room temperature and with an overpressure of 40-50 psi of hydrogen. Either 1 g of 10% Pd/C or 0.1 g of PtO_2 was used for every 100 g of sesquiterpene. The bomb was shaken until uptake of hydrogen ceased. The hydrogenation of valencene and premnaspirodiene was complete within two hours, while caryophyllene and isomerized caryophyllene mixtures typically required up to 48 hours to fully react. After hydrogenation was complete, the black reaction mixtures were then filtered through a celite pad. Valencane, premnaspirodiane, and caryophyllane were tested directly without further purification, while the isomerized mixtures were vacuum distilled (85-110 °C, 1 Torr) through a 10 in Vigreux column to isolate the hydrogenated sesquiterpenes as colorless oils.

Valencene (1). ^1H NMR (CDCl$_3$) δ: 5.33 (dt, J =4.9, 2.3 Hz, 1H, =CH), 4.68 (bs, 2H, =CH$_2$), 2.41-2.15 (m, 2H), 2.15-1.74 (m, 6H), 1.71 (t, J = 1.1 Hz, 3H, Me), 1.46-1.38 (m, 3H), 1.27-1.15 (m, 1H), 0.95 (s, 3H, Me), 0.88 (d, J = 6.4 Hz, 3H, Me). ^{13}C NMR δ 151.0, 143.4, 120.3, 108.5, 45.2, 41.3, 41.2, 38.1, 33.4, 32.9, 27.4, 26.1, 21.1, 18.6, 15.9

Valencane. ^1H NMR (CDCl$_3$) δ 1.78-1.55 (m, 3H), 1.47-1.05 (m, 10H), 1.05-0.89 (m, partial overlap, 2H), 0.86 (d, J = 6.6 Hz, 3H, Me), 0.85 (d, J = 6.7 Hz, 3H, Me), 0.78 (d, J = 6.5 Hz, 3H, Me), 0.69 (s, 3H, Me), 0.62 (t, J = 12.5 Hz, 1H). ^{13}C NMR δ 47.0, 43.8, 42.7, 39.1, 37.0, 33.4, 31.2, 30.2, 29.4, 29.3, 27.0, 20.3, 19.8, 15.4, 11.6. Anal. Calcd. for $C_{15}H_{28}$: C, 13.54; H, 86.46. Found: C, 13.67; H, 86.29.

Premnaspirodiene (2). ^1H NMR (CDCl$_3$) δ: 5.28 (bs, 1H, =CH), 4.72 (s, 1H, =CH$_2$), 4.68 (s, 1H, =CH$_2$), 2.59-2.37 (m, 1H), 2.12-1.94 (m, 1H), 1.93-1.49 (m, 12H), 1.74 (s, 3H, Me), 1.49-1.36 (m, 1H), 0.90 (d, J = 6.9 Hz, 3H, Me). ^{13}C NMR δ: 148.5, 139.2, 120.9, 108.1, 48.5, 46.8, 43.8, 37.7, 34.0, 32.8, 27.1, 22.0, 21.2, 20.1, 14.8

Premnaspirodiane. ^1H NMR (CDCl$_3$) δ: 1.85-1.70 (m, 1H), 1.66-0.95 (m, 11H), 0.90 (s, 3H, Me), 0.88 (s, 3H, Me), 0.85 (d, J = 6.6 Hz, 3H, Me), 0.84 (d, J = 6.6 Hz, 3H, Me). ^{13}C NMR δ: 49.0, 48.9, 43.7, 42.3, 41.9, 34.4, 33.8, 33.1, 32.4, 27.0, 24.7, 22.1, 22.0, 17.8, 17.6. Anal. Calcd. for $C_{15}H_{28}$: C, 13.54; H, 86.46. Found: C, 13.45; H, 86.55

Caryophyllene (3). ^{1}H NMR ($CDCl_3$) δ 2.60-1.84 (m, 7H), 1.78-1.37 (m, 5H), 1.62 (s, 3H, Me), 1.01 (s, 3H, Me), 0.99 (s, 3H, Me). ^{13}C NMR δ 154.9 (quat.), 135.7 (quat.), 124.6, 111.9, 53.9, 48.7, 40.6, 40.2, 35.0, 30.3, 29.6, 28.6, 22.9, 18.2, 16.5

Caryophyllane. Mixture of isomers: ^{1}H NMR ($CDCl_3$) δ 1.91-1.03 (m, 16H), 1.03-0.67 (m, 12H). Anal. Calcd. for $C_{15}H_{28}$: C, 13.54; H, 86.46. Found: C, 13.24; H, 86.77

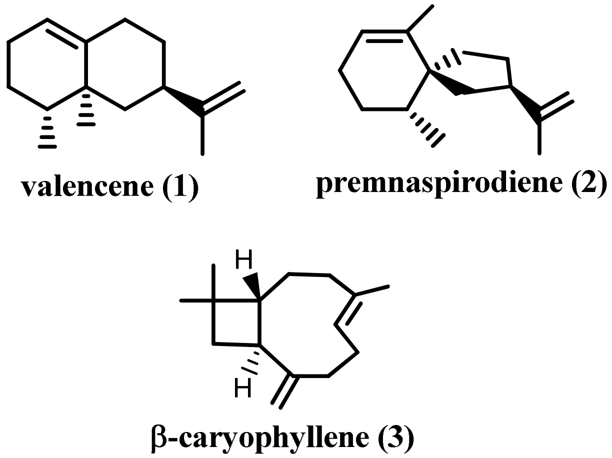
GC/MS Analysis. Samples (0.1 µL) of fluid (neat) were injected via syringe into a split/splitless injector set to a 50 to 1 split ratio. The injector was operated at a temperature of 325°C and a constant head pressure of 5 psi. The fuel sample was analyzed on a 30 m capillary column of 5% phenyl polydimethylsiloxane with a thickness of 1 µm. Initially, the oven temperature was maintained isothermally at 40°C for 4 min, followed by a 10°C/min ramp to 200°C, a 10 min hold at 200°C, a 10°C/min ramp to 300°C, and a 10 min hold at 300°C. All peaks were eluted by approximately 25 minutes although the analysis was allowed to run for 50 minutes. Each major chromatographic peak was identified with the assistance of the NIST Mass Spectral Database[31a], standard mass spectral analysis procedures,[31b,c] and on the basis of retention indices. All major components of the fluids could be identified with reasonable confidence due to the good separation achieved.

Structure Optimization and Total Eleectronic Energy Calculations. The sesquiterpene geometries were optimized with the General Atomic and Molecular Electronic Structure System (GAMESS) program.[32a] The geometries were optimized with second order Møller−Plesset perturbation theory (MP2)[32b] at a 6-31G(d,p) basis set using GAMESS.[32c] All of the calculations were done without imposing symmetry. Semi-numerical frequency calculations were run on all optimized geometries and no imaginary frequencies were found indicating that these are true minima. Total electronic energies were calculated using the zero point energy corrections from the semi-numerical frequency runs.

Results/Discussion:

Three multicyclic sesquiterpenes (valencene (**1**), premnaspirodiene (**2**), and caryophyllene (**3**)) were initially evaluated in this study (Figure 2). Valencene and premnaspirodiene were primarily of interest as they can be produced by a biosynthetic approach from glucose.[33] Valencene has a fused bicyclic structure reminiscent of decalin which has been considered as a high-performance jet fuel for military use.[34] Premnaspirodiene is also a bicyclic compound, but unlike the fused ring system of valencene, it has a unique spiro-linkage between the 6-membered and 5-membered ring system. β-caryophyllene was selected as a relatively cheap and abundant sesquiterpene that is isolated from clove oil and can be purchased commercially. Moreover, although the large scale biosynthesis of β-caryophyllene has not yet been demonstrated, the DNA sequence for β-caryophyllene synthase is known[35] and this sesquiterpene is a prime candidate for generation via a biosynthetic approach.

The first step to generate fuels from the sesquiterpenes was to hydrogenate the molecules to produce saturated hydrocarbons. Valencene, premnaspirodiene, and β-caryophyllene were hydrogenated with 10% Pd/C at moderate hydrogen pressures (40-50 psi) and at ambient temperature. The hydrogenation was carried out without the addition of solvent which simplifies the purification and


valencene (1) **premnaspirodiene (2)**

β-caryophyllene (3)

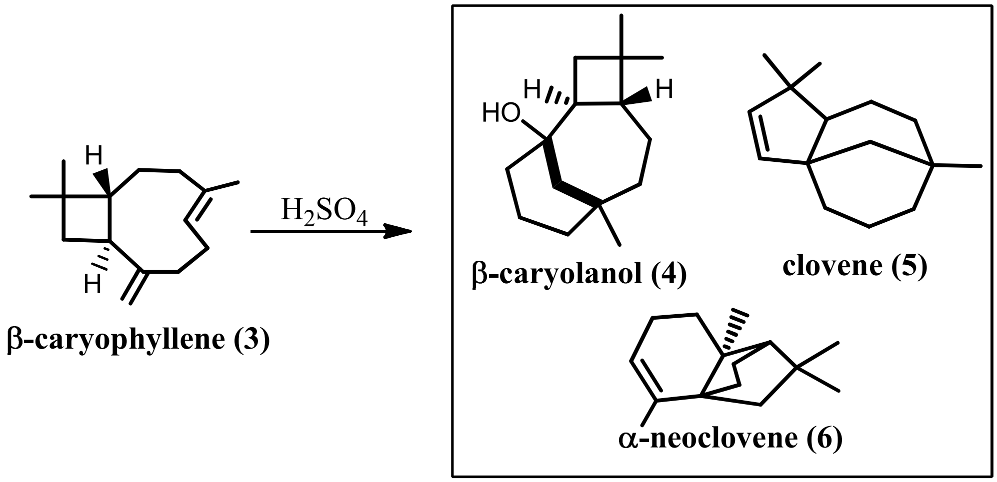
Figure 2. Structures of the two biosynthetic (valencene, premnaspirodiene) and one natural sesquiterpene (β-caryophyllene) studied in this work.

Table 1. Selected Properties of Pure Sesquiterpane Fuels

Properties	**H1**[a]	**H2**[a]	**H3**[a]	Diesel #2	Jet A
Density (g/cc)	0.88	0.88	0.85	0.85	0.82
NHOC (btu/gal)[b]	135,386	135,564	132,790	~129,000	~125,000
NHOC (MJ/L)	37.73	37.78	37.01	~36	~35
NHOC (MJ/kg)	42.92	42.83	43.54	~42	~42.5
-20 °C Viscosity (cSt)[c]	50.2	42.9	60.5	NA	<8.0
40 °C Viscosity (cSt)[c]	4.4	3.8	4.1	<4.1	
Cetane No.[d]	23.3	28.7	24.5	>41	

a. Fuel derived from the parent sesquiterpene by hydrogenation. b. Measured using ASTM D240N. c. Meaured using ASTM D445. d. Measured by IQT, ASTM D6890

carbon efficiency of the fuel synthesis process. In the case of **1** and **2**, racemic mixtures were obtained, however, in the case of **3**, two diasteriomers were observed in the ^{13}C NMR spectrum, consistent with the generation of two new stereocenters. The hydrocarbons were separated from the hydrogenation catalyst by filtration and purified by distillation under reduced pressure. The density, net heat of combustion (NHOC), cetane number, -20 °C and 40 °C viscosity, and flashpoint were measured for each of these fuels (Table 1). As expected based on the structures of the hydrocarbons, the volumetric NHOCs of these fuels were significantly higher than Diesel #2 and Jet A. However, the bicyclic ring structures also imparted higher viscosities to these fuels. Despite this limitation, both premnaspirodiane and the caryophyllanes have 40 °C viscosities within the specifications for Diesel #2. These fuels cannot be utilized as standalone jet fuels on the basis of their low temperature (-20 °C) viscosities which are significantly higher than the specification for Jet A. However, renewable fuels for military use are typically tested as 50:50 blends with conventional fuels. Further, recent studies on terpene dimer fuels suggest that acceptable low temperature viscosities can be achieved by blending sesquiterpane fuels with either conventional petroleum-based or other renewable fuels.[30] The spiro-linkage of premnaspirodiane resulted in the lowest viscosity, while the fused ring structures of the caryophyllanes had the opposite effect. The cetane numbers of the sesquiterpane fuels are not high enough for them to be used as conventional diesel fuels, however premnaspirodiane again performed better in this respect than the other two sesquiterpanes, exhibiting a modestly higher cetane number. In general, the relatively low cetane numbers of the sesquiterpanes are not surprising given the number of highly substituted carbons in each of the structures. These molecules each have 1 quaternary and 4 tertiary carbons which results in a significant increase in the ignition delay. One way to overcome this limitation

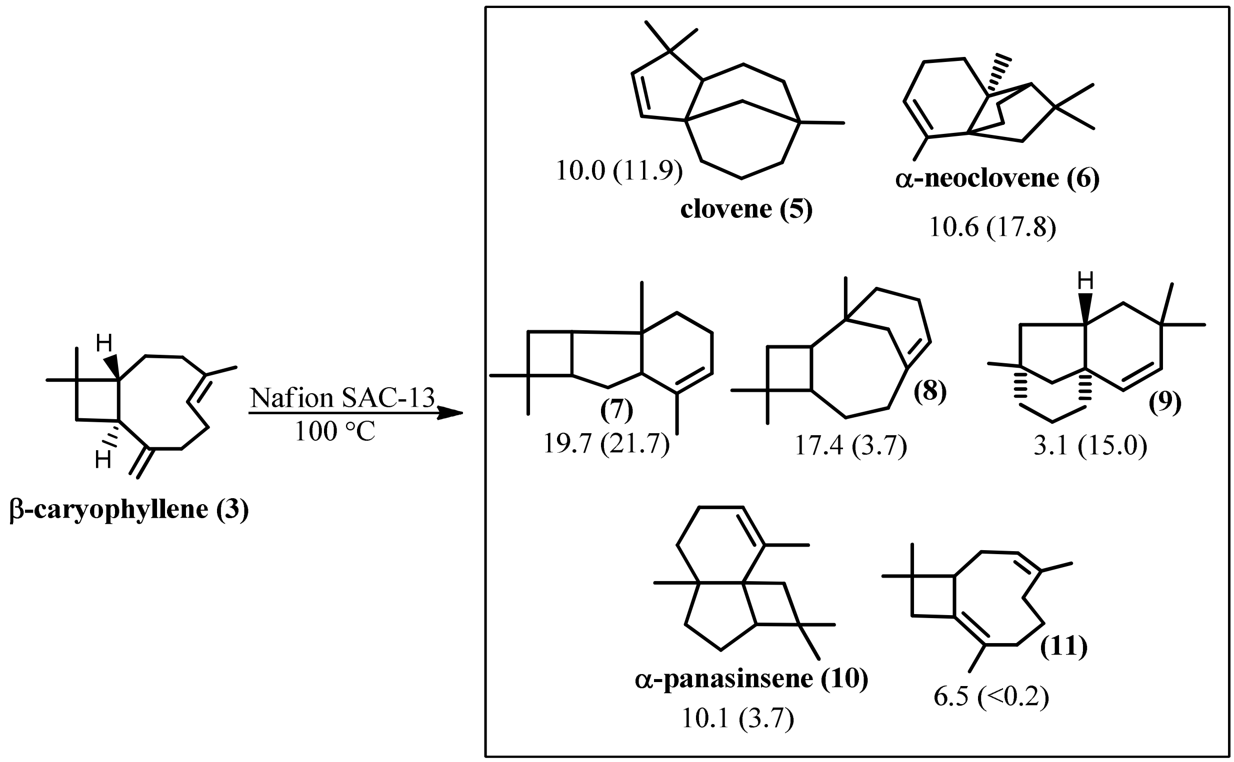


Scheme 1. Sulfuric acid catalyzed isomerization of β-caryophyllene

would be to prepare fuel blends that combine a high cetane component with the lower cetane multicyclic sesquiterpenes. SPKs and moderately branched ATJ fuels are potential blendstocks for this approach.[15,36,37]

After evaluating the initial set of multicyclic sesquiterpanes, it became of interest to explore additional structures that might have improved performance characteristics compared to those we had studied. Previous experience had shown the most important property for maximizing the net heat of combustion of a fuel is the density, with increased ring strain being a minor contributor.[38,39] Therefore, we became interested in α-neoclovene, a sesquiterpene with a density of 0.951 g/mL. Liquid hydrocarbons with such high densities have the potential to not only outperform conventional jet and diesel fuels, but also to have NHOCs that meet or exceed those of specialized missile fuels such as JP-10.[40] A survey of the literature revealed several classic studies focused on the acetic acid or sulfuric acid catalyzed isomerization of β-caryophyllene to primarily β-caryolanol (**4**), clovene (**5**) and neoclovene (**6**) (Scheme 1).[41] A particularly rigorous study of this work was conducted in 1995.[42] The authors reported that the reaction with sulfuric acid initially generated 18 hydrocarbons and 4 alcohols after 30 minutes. However, after three days, only three hydrocarbons and three alcohols remained. Regardless of the reaction time, one of the main products was β-caryolanol which comprised 44% of the total product after 30 min and 38% of the total product after 3 days. The hydrocarbon of interest for fuel applications, neoclovene, was present in 18% yield after the longer reaction time. The significant quantity of β-caryolanol generated in the sulfuric acid catalyzed process greatly limits the utility of this approach and this inspired the exploration of methods to reduce or eliminate the alcohol as a product.

The source of the alcohol is water present in the sulfuric acid and therefore it seemed logical to conduct the reaction with a heterogeneous acid catalyst in a dry environment. MMT-K10, an acid treated montmorillonite clay and Nafion SAC-13, a perfluorinated sulfonic acid resin supported on silica were initially chosen as promising candidates. The reaction with MMT-K10 was extremely vigorous and exothermic and generated a dark, viscous solution after only 15 min at 100 °C. This behavior was similar to that observed in a previous study on the reaction of MMT-K10 with β-pinene.[38] Hourly aliquots showed no change in the product distribution after the first hour, while GC analysis of the reaction mixture showed a complex distribution of monomers in a 6:4 ratio with dimers. Although not observed in the GC, heavier oligomers are also likely present. In light of the low selectivity of this catalyst, we abandoned it and examined Nafion SAC-13. Although this catalyst has much stronger acid sites than those present in MMT-K10, there are no Lewis acid sites suggesting that the behavior of this catalyst would be similar to that observed for sulfuric acid. Unlike sulfuric acid, Nafion SAC-13 has been shown



Scheme 2. Nafion SAC-13 catalyzed isomerization of β-caryophyllene. The first number under each structure is the area % obtained with low catalyst loading. The number in parentheses is the area % obtained with high catalyst loading. The remainder of the sesquiterpene distribution consists of unknown compounds. Sesquiterpenes represent 91.9 and 86.3% of the reaction mixture for the low catalyst and high catalyst reaction mixtures, respectively. The remainder is primarily composed of dimers ($C_{30}H_{48}$ compounds)

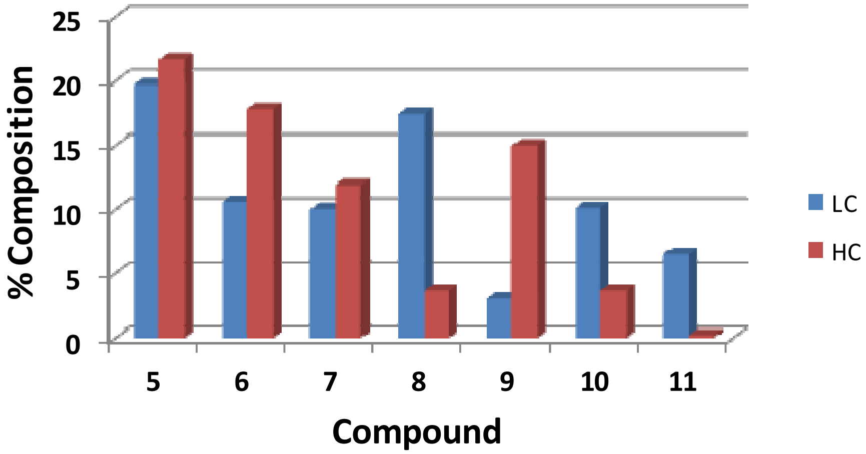


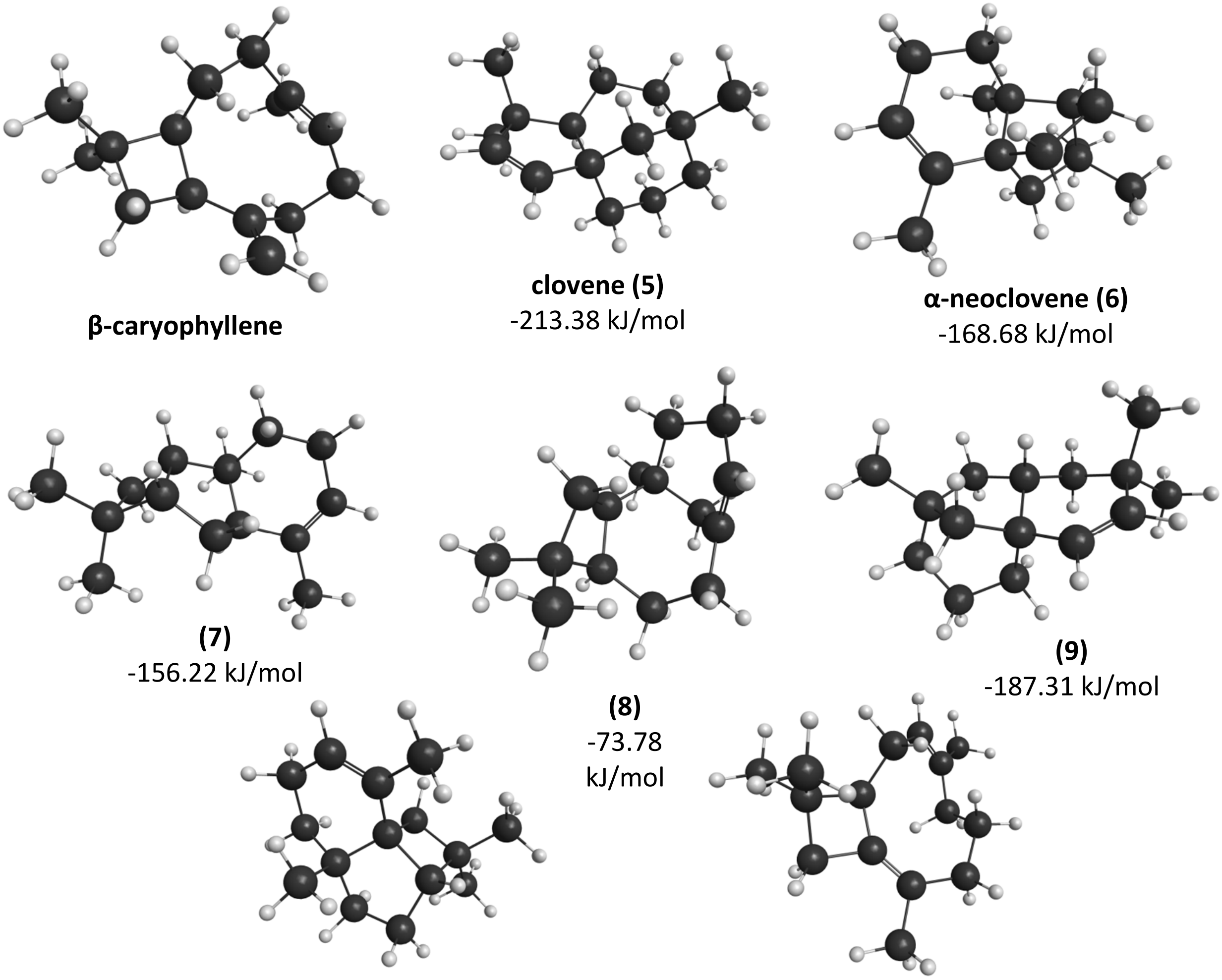
Figure 3. Isomer distribution resulting from the Nafion SAC-13 catalyzed isomerization of β-caryophyllene under high (HC) and low (LC) catalyst loadings.

to be readily recycled from alkene isomerization/dimerization reaction mixtures and does not poison hydrogenation catalysts in future steps.[14,39] The reaction with Nafion SAC-13 was allowed to proceed overnight at 100 °C with a catalyst loading of 1 g/100mL caryophyllene. This resulted in complete conversion of caryophyllene to a mixture of seven prominent sesquiterpenes (Scheme 2). In addition to the isomers, 13.7% of the reaction mixture was comprised of a complex distribution of dimers. In contrast to the sulfuric acid catalyzed isomerization, no alcohols could be detected in the product mixture. This result was confirmed by both GC/MS as well as FTIR spectroscopy. To determine the effect of reducing the catalyst loading, the reaction was also conducted for 24 h with 0.4 g catalyst/100 mL. The conversion of β-caryophyllene was still complete, but the distribution of sesquiterpene isomers was significantly altered (Figure 3) and the amount of dimer generated was reduced to 8.1% of the reaction mixture.

In addition to the lack of alcohols, there are other significant differences in the product distribution compared to that generated by sulfuric acid. First, **8** which was not reported as a product in the previous work is a significant component under the low catalyst protocol. This isomer is not too surprising given the structural similarity between it and β-caryolanol, the principal product generated in the sulfuric acid process. Other reports have shown that **8** can be generated from caryophyllene, either by hydrochlorination/dechlorination[43] or by reaction with mercury acetate.[44] Except for **9**, all of the other significant products including **5-7** are the principal hydrocarbon products generated with sulfuric acid.

The identities of the sesquiterpenes were established through a combination of GC/MS and ^{13}C NMR studies of the mixtures. 2D GC/MS that took into account both the mass spectrum and the retention time was utilized to make an initial structural assignment, while ^{13}C NMR of the complex mixture was utilized for corroboration. Excellent agreement was obtained for all of the principal products except for **9** which was a significant product under the high catalyst protocol. The GC/MS NIST library match for **9** was inconsistent with the NMR spectrum and required a more rigorous analysis of the NMR data. The olefinic region of the ^{13}C spectrum was characterized by two peaks of similar intensity at 127.3 and 132.8 ppm. The relative intensity of the peaks suggested that both resonances were from secondary sp^2-hybridized carbons. A DEPT NMR experiment confirmed that both resonances belonged to methines. Initially we believed that the unknown sesquiterpene was epi-clovene. However, the significant upfield shift of the unknown compound compared to clovene suggested that the sesquiterpene had a structure similar to pseudoclovene B which is a known product derived from the acid-catalyzed dehydration of β-caryolanol.[45] Moreover, epi-clovene is known to rearrange under acidic conditions to isoclovene.[46] No evidence for isoclovene was found in the NMR spectrum, suggesting that epi-clovene is not a likely candidate for the unknown sesquiterpene. Unfortunately, the ^{13}C NMR spectrum of epi-clovene has not been reported in the literature which precluded a direct comparison. However, a recent paper has reported the ^{13}C chemical shifts of pseudoclovene B (135.4 and 132.7 ppm)[47] and the olefinic carbons were observed at higher field than clovene (138.8 and 137.2 ppm).[42] Taking into account this evidence, we propose that sesquiterpene **9** is the epimer of pseudoclovene B. Attempts to isolate **9** by chromatography on silver nitrate doped silica were unsuccessful, but work is ongoing to isolate a pure sample and definitively characterize this product.

To provide more insight into the selectivity of the reaction for various isomerization products, we calculated the optimized geometries and semi-numerical frequencies to obtain the total electronic energy of each of the main components (Figure 4). Interestingly, **5** had the lowest energy of all the isomers followed by **9**, **6** and **7**. Compounds **11** and **8** had the highest energy and although these compounds were significant products at low catalyst loading, increased catalyst loading resulted in a significant decrease from 17.4% to 3.7% for **8** and 6.5% to less than 0.2% for **11**. α-panasinsene (**10**) also had a relatively high energy and higher catalyst loading again resulted in a decrease from 10.1 to 3.7%. These changes mirrored those of compound **9** with an increase of 12% and **6** with an increase of 7.2%. The other isomers were present in similar amounts under both high and low catalyst loadings. This result suggested that **8** and **10** were precursors to **9** and **6**, respectively. In support of this hypothesis, the acid catalyzed isomerization of **10** to **6** has already been proposed (Fitjer 1995), while the current



β-caryophyllene

clovene (5)
-213.38 kJ/mol

α-neoclovene (6)
-168.68 kJ/mol

(7)
-156.22 kJ/mol

(8)
-73.78 kJ/mol

(9)
-187.31 kJ/mol

α-panasinsene (10)
-126.91 kJ/mol

(11)
-25.85 kJ/mol

Figure 4. Optimized geometries and total energies relative to β-caryophyllene for acid-catalyzed isomerization products at the MP2/6-31G(d,p) level

study suggests that sesquiterpene **8** isomerizes to sesquiterpene **9** under high catalyst loadings. This result is also supported by the calculated energies, as **10** is 41.77 kJ/ mol higher in energy than **6**, while **8** is 113.53 kJ/mol higher in energy than **9**. It is also of interest that the product distribution is not directly correlated to the energies of the isomers. For example, compounds **6** and **7** are the most abundant isomers, despite having higher energies compared to **5**. In a similar fashion, calculated heats of formation favor **5** as opposed to **6** or **7**[42]. As expected this shows that the selectivity is dependent on the relative stability of the intermediate carbocations.

To evaluate the caryophyllene isomers as high density fuels, the mixtures were hydrogenated, purified by distillation, and some of their key fuel properties were measured (Table 2). Given the

Table 2. Properties of Hydrogenated Isomerized Fuel Blends

Properties	HDCL-8	HDCL-9	HDCL-10
Density (g/mL)	0.90	0.90	0.92[a]
NHOC (btu/gal)[b]	137,800	137,100	140,900
NHOC (MJ/L)	38.41	38.21	39.27
NHOC (MJ/kg)	42.68	42.46	42.68
-20 °C Viscosity (cSt)[c]	53.6	62.0	NM
Ignition Delay (msec)[d]	13.173	6.549	NM
Derived Cetane No.[d]	20.2	32.5	NM

a. Calculated using Advanced Chemistry Development (ACD/Lab) Software V11.02. b. Measured using ASTM D240N. c. Meaured using ASTM D445. d. Measured by IQT, ASTM D6890

conversion of caryophyllene to a variety of tricyclic structures, we expected the density of these mixtures to increase significantly with a subsequent increase in the net heat of combustion. As expected these fuel mixtures showed an increase in density of 0.05 g/mL (6%) over caryophyllane. The volumetric net heat of combustion also increased by 4 and 3% for the isomer mixtures achieved with high catalyst loading (HDCL-8) and low catalyst loading (HDCL-9), respectively. The density of potential fuel blends could potentially be further improved by taking distillate cuts. For example a fuel composed of only neoclovane (HDCL-10) would be expected to have a density of ~0.92 g/mL, with a calculated volumetric NHOC of nearly 141,000 btu/gal. The product distribution also affects other fuel properties including viscosity and cetane number. Although the low temperature viscosities of both blends were similar, HDCL-9 had a derived cetane number of 32 compared to 20 for HDCL-8. The most significant difference in the composition of the two blends is the greater concentration of hydrogenated **8** (**H8**) and significantly lower concentration of hydrogenated **9** (**H9**) in HDCL-9. Although not definitive, it appears that **H8** imparts a high cetane number while **H9** lowers the cetane number. Regardless, these results suggest that specific components of the blend may have a high cetane number which will allow for the isolation of a standalone high density diesel fuel. Moreover, it appears that the cetane number can be tuned to a certain extent by the reaction conditions.

Transitioning from caryophyllene, we investigated the isomerization of biosynthesized valencene and premnaspirodiene. Valencene was allowed to react with Nafion SAC-13 at 100 °C for 4-24 h. Analysis of the mixture after 4 h showed that **1** had isomerized to a complex mixture containing 9 sesquiterpenes. Only about 5% of the sesquiterpene fraction was unreacted **1**, while 5% of the reaction

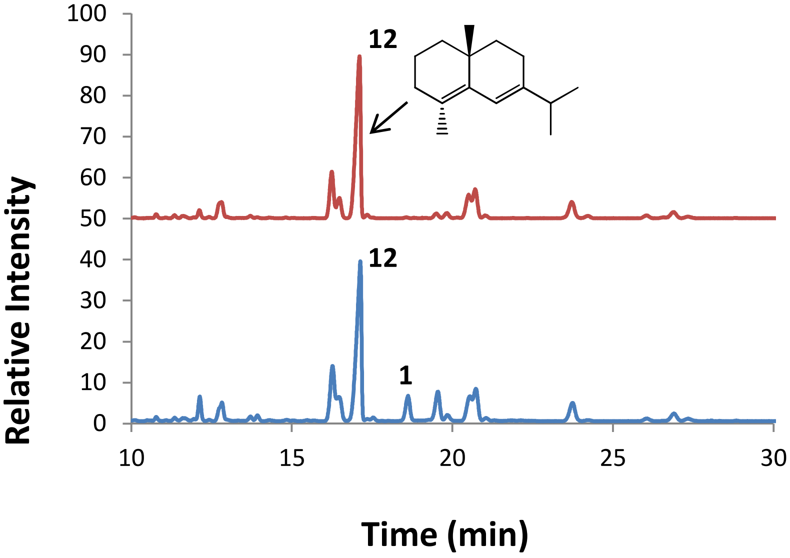


Figure 5. GC chromatograms of isomerized valencene (**1**) at 4 h (blue) and 24 h (red)

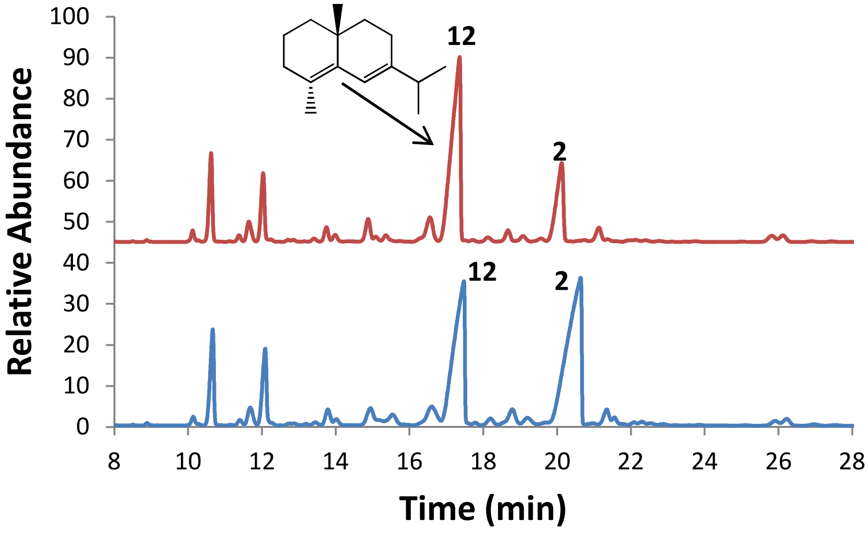
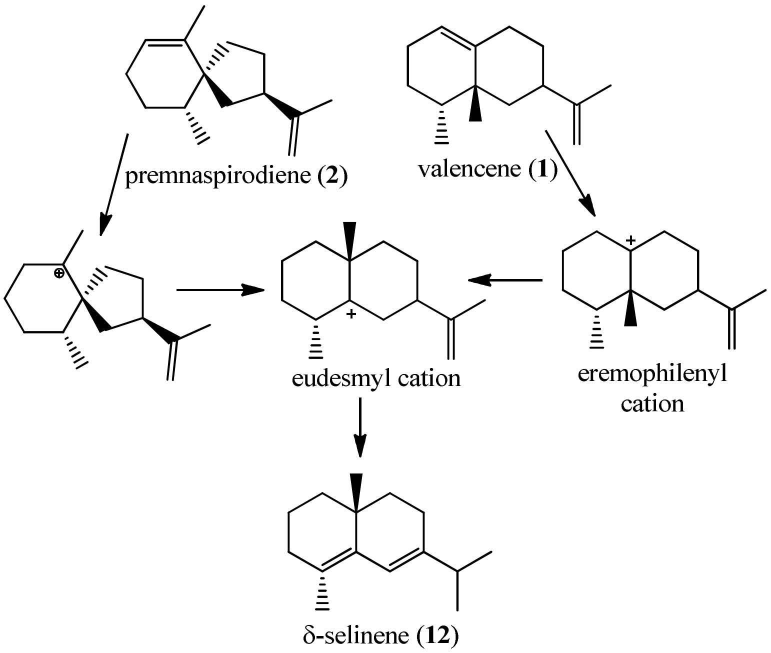


Figure 6. GC chromatograms of isomerized premnaspirodiene (**2**) after 4 h (blue) and 24 h (red)

mixture was composed of valencene dimers. The main product of the reaction was δ-selinene (**12**) which comprised 37% of the sesquiterpene fraction. After 24 h, less than 1% of the original valencene remained, while **12** comprised 46% of the sesquiterpenes (Figure 5) and dimers comprised about 10% of the reaction mixture. Although 2D GC/MS was utilized to characterize the other components of the mixture, no correlation was found between the library matches and the ^{13}C NMR data, with the exception of **1** and **12**. An analysis of the ^{13}C NMR spectrum revealed a total of 40 prominent peaks in the olefinic region of the spectrum (see Supporting Information), suggesting that all of the sesquiterpenes are bicyclic structures with two double bonds for each molecule.

In a similar fashion premnaspirodiene was isomerized to a mixture of five primary hydrocarbons. Again, **12** was a significant product, representing 28% of the mixture after 4 h and 44% after 24 h (Figure 6). The rate of premnaspirodiene isomerization was much slower than that for **1**, and **2** represented 37% of the product after 4 h and 14.7% after 24 h. No premnaspirodiene dimer was observed after 4 h and only about 1% was observed after the 24 h reaction. Similar to **1**, a total of 20 prominent peaks were observed in the ^{13}C NMR spectrum (see Supporting Information) suggesting that only bicyclic structures were formed in appreciable yield. The lack of tricyclic hydrocarbons derived from the isomerization of both **1** and **2** is not expected to improve the density or NHOC of product fuels compared to the hydrogenated native sesquiterpenes (Table 1). On this basis, no attempt was made to convert the isomerized mixtures to fuels.

The conversion of **1** and **2** to **12** is consistent with literature reports of related sesquiterpenes. Both valencene and premnaspirodiene are eudesmanes and rearrangement is expected to proceed through the eudesmyl carbocation (Scheme 3).[48,49] To provide a theoretical basis for the isomerization reaction we calculated the optimized geometries and total electronic energies of **1**, **2**, and **12** (Figure 7). Given that the isomerization of **1** was much more rapid than that of **2**, we expected **2** to have a significantly lower energy. Interestingly, this was not the case as **2** had an energy 36 kJ/mol higher than **1**. **12** was 86.59 and 50.49 kJ/mol lower in energy than **2** and **1**, respectively. The selectivity to **12** is not surprising given the calculated energies and taking into account other literature reports. For example, Bülow and König[50] reported that under acidic conditions Germacrene D isomerized primarily to the conjugated cadinenes zonarene and epizonarene with δ-selinene present as a significant product, particularly at long reaction times. Humulene has also been shown to isomerize primarily to δ-selinene in the presence of sulfuric acid.[51] In a recent paper Setzer[52] conducted an ab initio study that provided supporting evidence for the cyclization products of Germacrene D. Although there is an initial



Scheme 3. Conversion of premnaspirodiene and valencene to δ-selinene

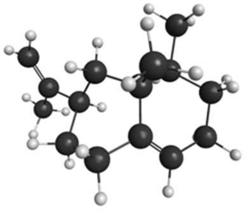
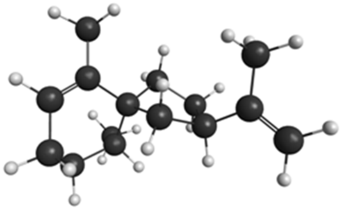
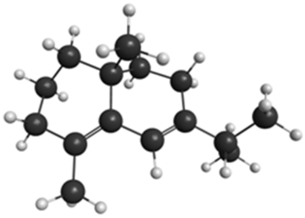
Optimized Structure	Compound Name	Energy relative to δ-selinene
	Valencene (**1**)	+50.49 kJ/mol
	Premnaspirodiene (**2**)	+86.59 kJ/mol
	δ-Selinene (**12**)	0 kJ/mol

Figure 7. Optimized geometries and relative electronic energies for valencene, premnaspirodiene, and δ-selinene at the MP2/6-31G(d,p) level

preference for cadinenes, this is likely due to the lower energy cadinenyl carbocations compared to selinyl carbocations, however, Setzer calculated that the eudesmane δ-selinene was the lowest energy isomer resulting from the rearrangement, with a 25 °C Gibbs free energy term 171.75 kJ/mol lower than Germacrene D.

Conclusions

High performance renewable fuels, whether generated by biological or chemical processes are synthetic fuels. Given the remarkable palette of hydrocarbons that nature and advances in metabolic engineering have provided for modern chemists, it makes sense that the next logical evolution in the development of renewable fuels would take advantage of the ability to generate hydrocarbons that have the potential to outperform petroleum-based fuels. To a certain extent, this has already taken place, as renewable fuels have been developed that burn cleaner than petroleum-based fuels and may have advantages such as high cetane numbers. In this work, we have demonstrated that renewable fuels with densities that exceed those of conventional jet fuels by up to 13% can be generated from multicyclic sesquiterpenes. This advance has the potential to improve the range of aircraft, ships, and ground vehicles without altering engine configurations. In addition, as strategies to efficiently convert lignocellulosic biomass into sugars improve and organisms are developed that can utilize these sugar mixtures and convert them to sesquiterpenes, these fuels can be produced on a scale that would help supplant significant quantities of petroleum.

In addition to high-performance fuels, the coupling of metabolic engineering, catalytic isomerization, and conventional synthetic organic chemistry has the potential to uncover new routes for the preparation of fine chemicals. For example, although the isomerization of valencene and premnaspirodiene did not result in structures that would likely lead to improved fuel properties, it did result in a formal synthesis of δ-selinene. This same type of strategy applied to other biosynthetic sesquiterpenes will allow for the synthesis of a wide variety of functional hydrocarbons that are rare and prohibitively difficult to isolate from plant extracts or prepare through a purely biosynthetic approach.

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Renewable multicyclic sesquiterpenes were converted into high density fuels with net heats of combustion up to 13% higher than Jet-A