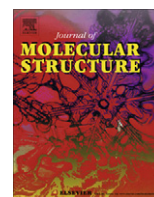




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Vibrational spectra and DFT calculations of squalene

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H I G H L I G H T S

- ▶ Squalene is an isoprenoid molecule with many bioactive properties.
- ▶ The infrared and Raman spectra of liquid squalene have been recorded and assigned for the principal bands.
- ▶ DFT computations were carried out to calculate the vibrational frequencies and spectral intensities.
- ▶ The structure including the bond distances was calculated for squalene.

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The isoprenoid compound squalene is a building block molecule for the production of essential cellular molecules such as membrane sterols, has several therapeutic activities including anticancer properties, and has commercial applications for a variety of industries including the production of cosmetics. While the physical structure of squalene has been known for many years, a spectroscopic understanding of the squalene molecular structure and how these spectrometric properties relate to the physical squalene structure has yet to be reported. In the present work we present the Raman and infrared spectra of liquid squalene, complemented by DFT calculations. The molecule has 234 vibrational frequencies and these have been categorized according to the different types of vibrational modes present. The vibrational modes are highly mixed and these have been assigned for the more prominent infrared and Raman bands.

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1. Introduction

Squalene (Fig. 1) is a linear triterpene molecule found in many organisms throughout nature including mammals, plants, and bacteria [1,2]. Organisms primarily utilize squalene by cyclizing it to form a vast array of triterpenes that have functions ranging from regulating membrane fluidity to protection against pathogens [3,4]. Squalene also exists in organisms in its native, linear state and can be found in high quantities in the oils of human skin, shark liver, and the oil of several plants such as olive [5–8].

Studies have suggested that squalene has many bioactive properties including antioxidant and anticancer activities, and these properties may contribute to the health benefits of the Mediterranean diet [1,9,10]. This diet is high in squalene-rich olive oil and has a low occurrence of associated cancers [11–13]. Squalene has also been shown to reduce the prevalence of colon cancer resulting from chemical exposure and breast cancer caused by DNA damage [1,14–16]. Interestingly, sharks are known to not have cancer and

this is thought to be related to the high level of squalene in sharks [17]. Squalene is also used for pharmaceutical and commercial purposes such as an emulsifier for drug delivery, an adjuvant for vaccine delivery, a moisturizer in cosmetics, a surfactant in dry cleaning, and as a vulcanization test molecule [18].

The biosynthesis of squalene utilizes the isoprenoid pathway [4]. Two molecules of the C₁₅ isoprenoid intermediate farnesyl diphosphate (FPP) are condensed to form C₃₀ squalene [19,20]. This reaction is carried out by the enzyme squalene synthase, requires the reducing agent NADPH, and results in the connection of two farnesyl moieties from C1 of one farnesyl molecule to C1 of the second farnesyl molecule [19,20]. This results in the linear structure of squalene with six carbon–carbon double bonds (C=C) in the backbone at carbons 2, 6, 10, 14, 18, and 22 (Fig. 1) that should provide unique qualities for spectroscopic studies such as Raman spectroscopy.

Reports of Raman spectroscopy analysis for squalene are limited and have been used to identify squalene as a component of olive oil [21], to study the effect of squalene on the packing of lipid bilayers and monolayers [22], to analyze the degradation of squalene by bacteria [23], and to analyze the components of sebaceous gland exudates [24]. However, there are no reported studies

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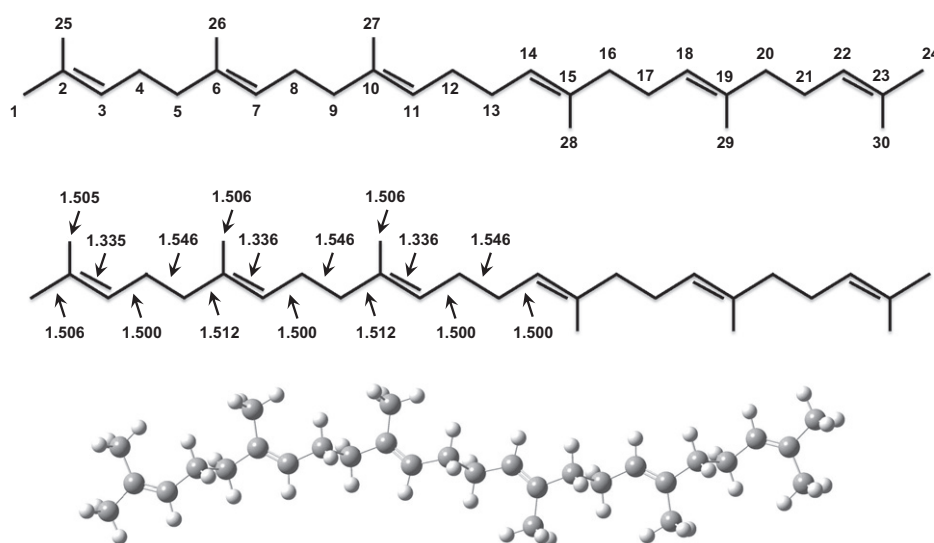


Fig. 1. The structure of squalene. The calculated bond distances (Å) are shown in the center.

on a detailed analysis of the squalene structure based on Raman spectroscopy. Given the importance of this molecule, a molecular-level understanding of the squalene structure will be invaluable in advancing squalene functional studies.

We recently utilized Raman spectroscopy to study the structure of hydrocarbons called botryococcenes from the green microalgae *Botryococcus braunii* that are structurally and biosynthetically (produced from two farnesyl molecules) similar to squalene [25]. Botryococcenes have several Raman active C=C bonds including backbone C=C bonds similar to squalene, a central branch C=C bond, and exomethylene groups [26]. Coupled with DFT calculations, Raman spectroscopy was able to distinguish between these different C=C groups; backbone C=C frequencies in the 1663–1679 cm^{-1} range, exomethylene frequencies in the 1646–1655 cm^{-1} range, and branch C=C frequencies in the 1642–1649 cm^{-1} range [25]. While squalene is similar to botryococcenes, it only contains backbone C=C groups. Here we report a similar analysis of squalene using Raman spectroscopy as well as infrared spectroscopy to assign frequencies to all bonds in squalene. In addition, density functional theory calculations were carried out to complement the experimental work.

2. Experimental methods

A Jobin–Yvon U-1000 spectrometer equipped with a liquid nitrogen-cooled charged-coupled device (CCD) detector was used to record the liquid Raman spectrum of squalene. A Coherent Verdi-V10 laser operating at 532 nm was used and typically operated at 2 Watts of power. The liquid mid-infrared spectrum of squalene was recorded on a Bruker Vertex 70 FT spectrometer equipped with a globar light source, a KBr beamsplitter and deuterated lanthanum triglycine sulfate (DLATGS) detector. Liquid squalene was obtained from Sigma.

3. Theoretical computations

Density functional theory (DFT) computations were carried out using the B3LYP/cc-pvtz basis set. Squalene can possess a variety of conformations resulting from internal rotations about their single C–C bonds. Hence, calculations were carried out on several conformations of the molecule but only minor effects on the vibrational frequencies and bond distances were found. A scaling factor of 0.969 was used for all frequencies above 1350 cm^{-1} [25] and 0.980 was used for the lower frequencies.

4. Results and discussion

The calculated structure of squalene (Fig. 1-center) has bond distances in line with expectations. The C=C bond distances are all 1.336 ± 0.001 Å, the C–C single bonds not adjacent to the double bonded carbon atoms are 1.546 ± 0.001 Å, and single bonds adjacent to the double bonded carbon atoms are 1.506 ± 0.006 Å. Figs. 2 and 3 show the experimental liquid-phase Raman and infrared spectra of squalene, respectively. The computed spectra for the individual (vapor phase) molecule are also shown in the figures. The frequency agreement between the experimental and calculated spectra is in good agreement, but as is commonly the case, the intensities are in somewhat poorer agreement. Since squalene has 234 vibrational frequencies, both the C–H stretching region (50 vibrations in the 2800–3100 cm^{-1} region) and the fingerprint region (184 vibrations below 1700 cm^{-1}) are extremely rich with spectral bands. This makes it very difficult to do a one to one correlation between observed and calculated spectral bands. The calculated frequencies are expected to be within 10 cm^{-1} of the

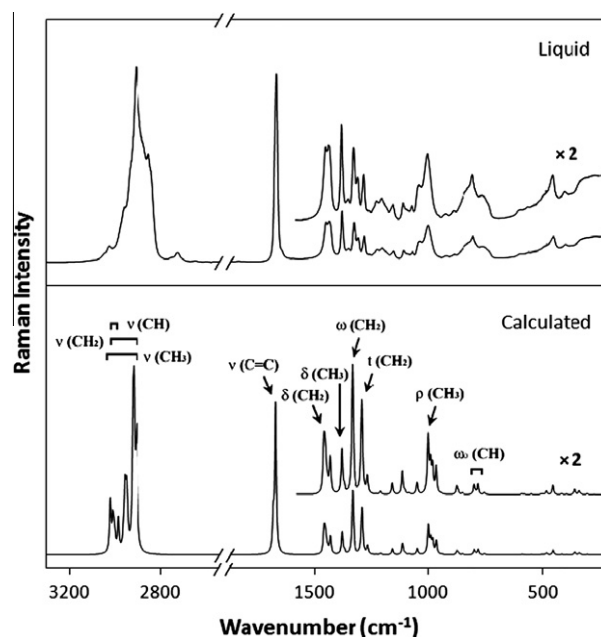


Fig. 2. The experimental and calculated Raman spectra of liquid squalene.

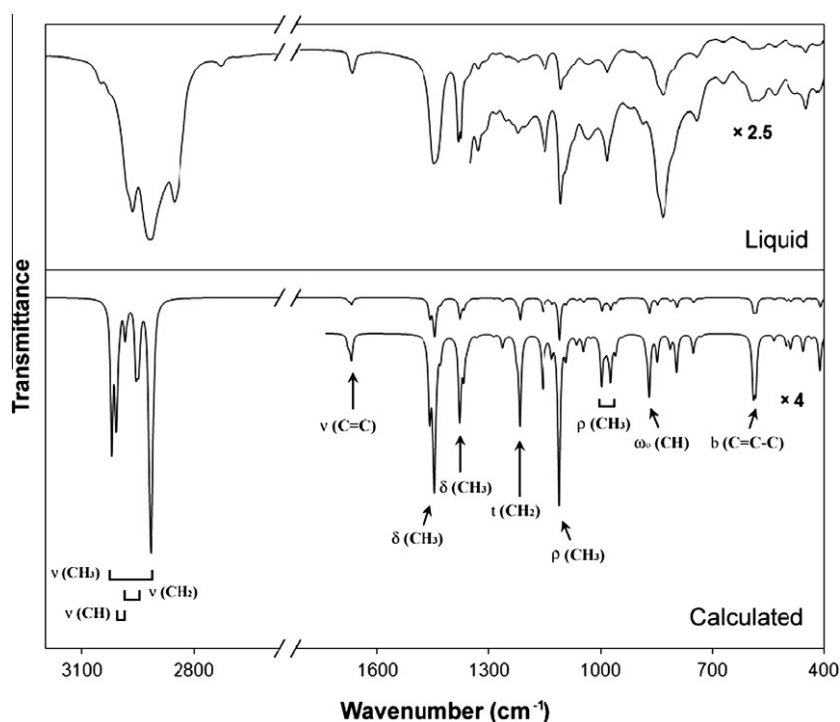


Fig. 3. The experimental and calculated infrared spectra of liquid squalene.

Table 1
Vibrations of squalene.

Symbol	Vibration	Wavenumber range (cm ⁻¹)	Number of vibrations
ν(CH ₃)	CH ₃ stretch	2911–3024	24
δ(CH ₃)	CH ₃ deformation	1254–1460	24
ρ(CH ₃)	CH ₃ rock	943–1228	16
τ(CH ₃)	CH ₃ torsion	110–209	8
ν(CH ₂)	CH ₂ stretch	2904–2988	20
δ(CH ₂)	CH ₂ deformation	1428–1460	10
ω(CH ₂)	CH ₂ wag	1207–1333	10
τ(CH ₂)	CH ₂ twist	1012–1291	10
ρ(CH ₂)	CH ₂ rock	730–1207	10
ν(CH)	=C–H stretch	3006–3012	6
ω _i (CH)	CH wag (in-plane)	1012–1379	6
ω _o (CH)	CH wag (out-of-plane)	778–1000	6
ν(C=C)	C=C stretch	1668–1679	6
ν(C–C)	C–C stretch	730–1379	23
b(C–C–C)	C–C–C angle bend	169–503	6
b(C=C–C)	C=C–C angle bend	319–592	12
ω _o (C–CH ₃)	C–CH ₃ wag (out-of-plane)	452–503	8
ω _i (C–CH ₃)	C–CH ₃ wag (in-plane)	353–434	8
τ(C–C)	Internal rotation (C–C)/Skeletal	4–209	15
τ(C=C)	Internal rotation (C=C)/Skeletal	42–209	6

observed frequencies, but since there are often several observed spectral bands within 10 cm⁻¹ of each other, identifying which correlates to which calculated vibrational frequency is challenging. Rather than list all of the calculated frequencies for squalene in the text here, these are available in the [Supplementary material as Table S1](#). It should be noted that the recorded spectra are for the liquid phase where intermolecular interactions greatly affect vibrational frequencies below about 200 cm⁻¹. Such interactions cannot be accounted for with our DFT calculation for non-interacting individual molecules. [Table 1](#) presents a summary of the

Table 2
Observed and calculated frequencies for the more prominent bends of squalene.

Frequency (cm ⁻¹)			Approximate assignment
Calculated ^a	Infrared ^b	Raman ^b	
2960 (97, 0)	2967 vvs		ν(CH ₃), ν(CH ₂)
2919 (1613, 4)	2920 vvs		ν(CH ₃), ν(CH ₂)
2919 (5, 117)		2913 vvs	ν(CH ₃), ν(CH ₂)
1670 (13, 0)	1668 m		ν(C=C)
1668 (0, 100)		1668 vvs	ν(C=C)
1458 (0, 12)		1451 ms	δ(CH ₃), δ(CH ₂)
1446 (58, 0)	1449 vs		δ(CH ₃), δ(CH ₂)
1379 (42, 0)	1383 s		δ(CH ₃), ω _i (CH), ν(C–C)
1379 (0, 12)		1382 s	δ(CH ₃), ω _i (CH), ν(C–C)
1333 (0, 29)		1330 ms	δ(CH ₃), ω _i (CH ₂), ω _i (CH)
1287 (0, 15)		1281 m	δ(CH ₃), τ(CH ₂)
1218 (52, 0)	1224 m		ρ(CH ₃), τ(CH ₂), ν(C–C)
1157 (31, 0)	1151 m		ρ(CH ₃), τ(CH ₂), ω _i (CH)
1112 (100, 0)	1108 ms		ρ(CH ₃), ν(C–C)
1000 (0, 12)		1003 ms	ρ(CH ₃), ω _o (CH)
998 (27, 0)	984 m (broad)		ρ(CH ₃), ν(C–C)
975 (26, 0)			ρ(CH ₃), ν(C–C)
872 (35, 0)	836 ms (broad)		ω _o (CH), ν(C–C)
850 (16, 0)			ω _o (CH), ν(C–C)
796 (23, 0)			ω _o (CH), ν(C–C)
795 (0, 3)		804 m	ω _o (CH), ν(C–C)
452 (0, 3)		454 m	ω _o (C–CH ₃), b(C=C–C)

^aRelative intensities calculated for infrared and Raman bands are given in parentheses (IR, R).

^bs-Strong; m-medium; v-very.

different types of vibrational modes in squalene and the frequency ranges calculated (and observed) for each. [Table 2](#) presents a condensed version showing the observed and calculated frequencies and intensities along with approximate descriptions for the more prominent infrared and Raman bands. It should be noted that for a large molecule such as this, the vibrational modes, especially

for the low-frequency ones, will be highly coupled. Hence a single vibrational frequency, for example, may have contributions from C—C stretchings, CH wags, CH₃ rocks, as well as skeletal motions. The specific calculated frequencies are in Table S1. Some of the vibrational descriptions based on the symbolic representation in Table 1 are shown in Figs. 2 and 3.

In our previous study of the botryococcene hydrocarbons we focused our attention on the C=C stretching modes and found that each bond resulted in an individual vibrational frequency for the most part without significant interactions with the stretching of other bonds [25]. We found that the backbone, exomethylene, and branch C=C frequencies gave different frequency ranges. For squalene we find that the two C=C groups near the ends of the molecule (carbons 2 and 22) vibrate together (symmetrically or antisymmetrically) giving rise to two almost identical frequencies of 1679 cm⁻¹ (Table 1). The other four C=C bonds (carbons 6, 10, 14, 18) vibrate simultaneously with differences in the phase of the motions, and these produce four frequencies in the 1668–1672 cm⁻¹ range (Table 1). For the botryococcene molecules we previously found that the backbone C=C vibrations were in the 1663–1679 cm⁻¹ range [25], so our results here for squalene are consistent with that of botryococcenes.

In addition to the intense C=C stretching bands in the Raman we observed several other bands with strong intensity. These include all types of C—H stretchings, the CH₃ deformations $\delta(\text{CH}_3)$, CH₂ deformations $\delta(\text{CH}_2)$, CH₂ wags $\omega(\text{CH}_2)$, CH₂ twists $t(\text{CH}_2)$, and CH₃ rocks $\rho(\text{CH}_3)$. In the infrared spectrum the C=C stretching bands are considerably less intense, but other bands show up with strong intensity including the C—H stretches, the CH₃ deformations $\delta(\text{CH}_3)$, CH₂ twists $t(\text{CH}_2)$, and CH₃ rocks $\rho(\text{CH}_3)$.

5. Conclusions

Squalene is an important molecule because of its bioactive properties and similarity to the botryococcenes which we studied previously [25]. We have recorded its Raman and infrared spectra and used DFT computations to predict its structure and vibrational frequency. The match between theory and experiment is excellent. However, the molecule has 234 vibrations, many of which are strongly coupled, so the descriptions are very complicated.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.molstruc.2012.10.008>.

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Table S1.

Calculated frequency (cm ⁻¹)	Relative intensity (Infrared, Raman)	Symbol		
4	(0, 0)		τ(C-C)	
8	(0, 0)			
13	(0, 3)			
15	(0.1, 0)			
19	(0, 2)			
22	(0, 0)			
26	(0, 1)			
30	(0, 0)			
36	(0.2, 0)			
38	(0, 1)			
42	(0, 2)		τ(C=C)	
47	(0, 1)			
48	(0, 0)			
54	(0, 0.3)			
57	(0.1, 0)			
65	(0.4, 0)			
67	(0, 0.4)			
70	(0.1, 0)			
87	(0, 1)			
106	(0.2, 0)			
110	(0, 3)		τ(CH ₃)	
118	(0, 1)			
132	(0.4, 0)			
135	(0.2, 0)			
149	(0, 0.3)			
150	(0.1, 0)			
154	(0, 0.3)			
169	(0, 2)			
181	(0.2, 0)			
188	(0.5, 0)			
192	(0, 0.4)		τ(C=C)	
208	(0, 0)			
209	(0.1, 0)			
268	(0, 1)			
277	(0, 0)			
282	(1, 0.2)			
294	(0, 0)			
299	(0, 3)			
319	(2, 0)			
325	(0, 1)			
329	(1, 0)		b(C-C-C)	
334	(0, 9)			
337	(0.1, 0)			
353	(0, 13)			
383	(2, 0)			

