

## Solid-State $^{13}\text{C}$ Chemical Shift Tensors in Terpenes. 3. Structural Characterization of Polymorphous Verbenol

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**Abstract:** Solid-state NMR analyses demonstrate that verbenol is a mixture of polymorphic materials containing two distinct lattice types. The major crystalline component, characterized by X-ray, consists of three molecules per asymmetric unit. The minor form contains four molecules per asymmetric unit but crystals suitable for X-ray analysis are unavailable. The minor polymorph is metastable and may be converted to the major form by melting and cooling to resolidify. Experimental and ab initio computed chemical shift tensors are compared to demonstrate that most of the structural variations in both polymorphs arise from differences in the orientation of the hydroxy hydrogen. Intramolecular steric interactions partially restrict allowed hydroxy hydrogen dihedral angle orientations. For the major form, comparison of experimental and various theoretical tensor principal values further narrows this dihedral angle to the range between  $45^\circ$  and  $80^\circ$ . Hence, tensor predictions generally agree with X-ray determined angles of  $55^\circ$ ,  $69^\circ$ , and  $74^\circ$  for the hydroxy dihedral angle. This tensor analysis is also applied to the minor form, but with less available information, only plausible structures can be proposed. Utilizing both the steric restrictions and principal and isotropic shift values, it is suggested that the minor form may involve molecules with dihedral angles near either  $60^\circ$  or  $180^\circ$ . Previous studies on similar compounds show that such a lattice could consist of either a ring or a continuous repeating helical structure.

### Introduction

NMR spectroscopy has proven to be a powerful and general method for determining molecular structure and dynamics. In solids, NMR analysis is often complicated by factors not found in solutions. Particularly interesting is the occurrence of polymorphism in the solid state. Polymorphism in a solid is the occurrence of different packing arrangements for the same molecules.<sup>1</sup> Such solids may differ appreciably in molecular conformations and lattice structures depending on the method of preparation. The frequency of occurrence of polymorphism in organic solids is difficult to quantify and one author has stated “every compound has different polymorphic forms...and the number of forms known for a given compound is proportional to the time spent in research on that compound”.<sup>2</sup> Most X-ray and solid-state NMR studies tend to focus only on a single polymorphic form.<sup>3</sup>

Interestingly, many molecular solids are composed of multiple conformations of similar energies. These conformational isomers form either multiple conformationally distinct lattice types in a solid mixture or crystallize with two or more conformations within a single unit cell. This latter case yields single crystals containing multiple molecules in the asymmetric unit. Such

crystals are readily characterized by diffraction methods. It has been determined that 8.3% of all compounds<sup>4</sup> and 40% of alcohol crystals<sup>5</sup> in the Cambridge database have more than one molecule in the asymmetric unit. However, even the availability of a X-ray structure from a single crystallite is no guarantee that the bulk solid does not contain a mixture of polymorphic forms. Crystallographers typically select the best crystal from a mix and thus may choose only one representative of a set of mixed crystallites. Evidence for this proposal is found in solid 1,3,5-triamino-2,4,6-trinitrobenzene. The published crystal structure is centrosymmetric but the bulk solid exhibits second-order harmonic generation, a property of noncentrosymmetric solids.<sup>6</sup> One explanation is that the crystal used in diffraction was atypical of the bulk solid.

Evidence for mixtures of polymorphs in solids is found in  $^{13}\text{C}$  solid-state NMR spectra by the presence of multiple isotropic lines for each carbon in a given structure. Lines arising from a single lattice type display common peak intensities and may exhibit more than one line per carbon corresponding to multiple molecules per asymmetric unit. Additional lattice forms present may exhibit lines of different intensities as an alternative arrangement may have a different stability. Relatively few NMR data have been reported on such mixed polymorphic solids. However, the frequent and perhaps underestimated<sup>7</sup> occurrence of such solids makes it valuable to investigate what contributions NMR can make. Furthermore, such solids are unsuitable for diffraction studies but have inherent NMR interest as they provide challenging cases for evaluating new NMR capabilities and chemical shift computational correlations of these data.

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Analysis of polymorphic mixtures using the chemical shift tensor is especially attractive as such tensors have been shown to be extremely sensitive to relatively minor variations in the molecular structure.<sup>8,9</sup> In addition, excellent experiments are currently available for measuring principal values in powdered microcrystalline solids.<sup>10</sup> We have recently demonstrated the value of shift tensors as a way to assign the majority of isotropic resonances to the two molecules in the asymmetric unit of parthenolide<sup>9</sup> by comparing ab initio computed tensors with PHORMAT and FIREMAT derived experimental principal values.

Solid-state verbenol is analyzed herein using the FIREMAT method.<sup>11</sup> This solid contains up to six different isotropic lines per carbon. High-quality computed tensors for various test conformations are compared with experimental tensors to demonstrate that the observed differences arise mainly from variation in the dihedral angle of the hydroxy hydrogen in verbenol. Most probable dihedral angles in the solid state are described for the major polymorph based on model structures and compared to the crystal structure to verify the reliability of these methods in the study of polymorphism. Finally, analysis of a second minor form without a known X-ray structure is described and likely molecular conformations described.

## Experimental Section

The 2D INADEQUATE spectrum was acquired on 102 mg of verbenol dissolved in 0.7 mL of  $\text{CDCl}_3$ . Analysis was performed at 26 °C using a Varian 500 INOVA spectrometer with a 5 mm probe operating at a frequency of 125.638 MHz. Other analysis parameters include spectral widths of 22.547 kHz in both dimensions, a 90°  $^{13}\text{C}$  pulse of 16  $\mu\text{s}$ , a recycle time of 8.6 s, and a decoupler frequency of 499.617 MHz. A total of 64 evolution increments of 64 transients each were collected for a total analysis time of 19.6 h. Digital resolutions of 0.31 and 352.30 Hz per point were acquired in the acquisition and evolution directions, respectively. This liquid spectrum was referenced to the central line of  $\text{CDCl}_3$  at 77.23 ppm. Processing and peak detection were performed using software described previously<sup>12</sup> with all bonds established at greater than the 99.99% confidence level.

Three separate FIREMAT analyses were performed on solid verbenol. Two analyses involved the major polymorph alone. These experiments differed only in the decoupler frequency, i.e., 400.1212 and 400.1232 MHz, to optimize the proton decoupling of aliphatic and olefinic protons, respectively. All analyses were performed on a Chemagnetics CMX400 NMR using a 7.5 mm PENCIL probe and operating at a frequency of 100.619019 MHz. Other parameters include a contact time of 3 ms, spinning speed of 440 Hz,  $^1\text{H}$  and  $^{13}\text{C}$  90° pulses of 4.1 and 4.3  $\mu\text{s}$ , respectively, spectral widths of 14.080 and 84.480 kHz in the evolution and acquisition dimensions, respectively, a pulse delay of 4 s, and 32 evolution increments of 384 transients each. A digital resolution of 11.0 Hz per point was acquired for the acquisition dimension. The FIREMAT data replication and subsequent rearrangement yields a significant increase in evolution points over the actual number acquired,<sup>11</sup> thus a digital resolution of 10.7 Hz per point was obtained for the evolution dimension. All FIREMAT data were TIGER processed.<sup>13</sup>

The third FIREMAT analysis was performed on a solid containing both the major and minor polymorphs in a 0.68/0.32 mixture. This

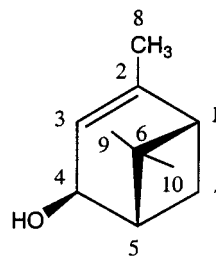


Figure 1. Structure of verbenol.

analysis was performed at a  $^{13}\text{C}$  frequency of 100.618956 MHz and a  $^1\text{H}$  decoupler frequency of 400.12325 MHz. Other parameters are as described above.

One-dimensional  $^{13}\text{C}$  isotropic spectra of solid verbenol, required for TIGER processing, were obtained directly from the FIREMAT data. Specifically, the evolution points associated with the first acquisition points of each rotor period were selected, using the replication procedure of Gan,<sup>11,14</sup> and separately processed to yield the required isotropic guide spectrum. These spectra had digital resolutions and spectral widths of 10.7 Hz per point and 14.080 kHz, respectively.

The variable contact time CP/MAS analysis was performed using a total of 40 contact times varying in 5  $\mu\text{s}$  steps from 5 to 20  $\mu\text{s}$ , 10  $\mu\text{s}$  steps from 30 to 100  $\mu\text{s}$ , 100  $\mu\text{s}$  steps from 100 to 900  $\mu\text{s}$ , and 1 ms steps from 1 to 20 ms. Analyses were performed on a Chemagnetics CMX100 spectrometer operating at 25.152 MHz with a 9.5 mm probe. All analyses were performed using a spectral width of 20.0 kHz, 256 transients, an acquisition delay of 5 s, a 90°  $^1\text{H}$  pulse of 4.0  $\mu\text{s}$ , and a  $^1\text{H}$  decoupler frequency of 100.0198 MHz. A digital resolution of 4.8 Hz per point was acquired for all spectra. The 2D exchange experiment was performed using identical parameters except a contact time of 3.0 ms and a mixing time of 100 ms were used, with 1000 transients collected.

Powder diffraction data were collected on a verbenol sample containing both the major and the minor forms using  $\text{Cu K}\alpha$  radiation of wavelength 1.5409 Å on a Rigaku uniflex powder X-ray diffractometer with  $2\theta/\theta$  scans within  $0 \leq 2\theta \leq 55^\circ$ .

Computations of tensor shielding values were performed with parallel processing using Gaussian 94 running on IBM SP computers. Computed shieldings were converted to shifts using slope and intercept values previously established for similar terpenes.<sup>9,15</sup> Specifically, respective slope and intercept values of 1.03 and 190.4 for  $\text{sp}^3$  carbons and 1.01 and 194.9 for  $\text{sp}^2$  carbons were used.

Verbenol was obtained as a 0.68/0.32 mixture of the major/minor polymorphs from ICN and as a 0.92/0.08 mixture from Aldrich. Polarimetry in acetone established that the ICN material had an enantiomeric content of 0.6/0.4 1S,4S,5S/1R,4R,5R. The pure crystalline form of the major polymorph was prepared by slow evaporation from methanol. All attempts to obtain a pure sample of the minor polymorph by evaporation from a variety of solvents as well as cooling a melt failed. Further, visual recognition and mechanical separation of the two forms were also unsuccessful. Thus, crystals of the minor form are presently unavailable for X-ray analysis.

## Results and Discussion

### Evidence of Mixed Polymorphic Forms in Solid Verbenol.

Verbenol is a monoterpene alcohol with the molecular structure shown in Figure 1. Most studies of verbenol have emphasized its pheromonal properties and a crystal structure was only recently reported from our laboratory.<sup>16</sup> An isotropic  $^{13}\text{C}$  NMR spectrum of solid verbenol revealed the presence of several lines per carbon with 36 resolved lines present for the 10 distinct carbons found in verbenol. While most carbons showed multiple lines, the  $\text{sp}^2$  carbons were especially dispersed, splitting into

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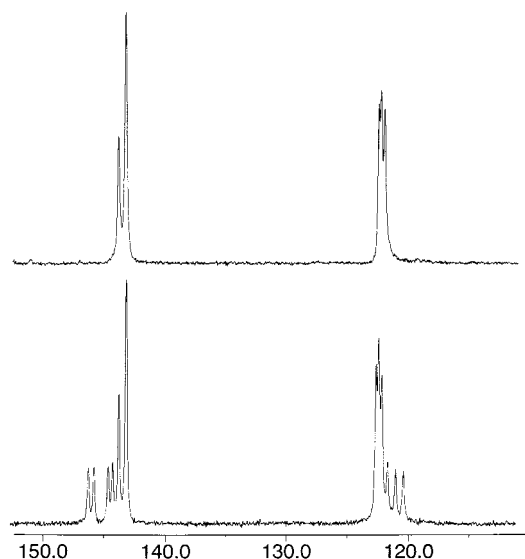
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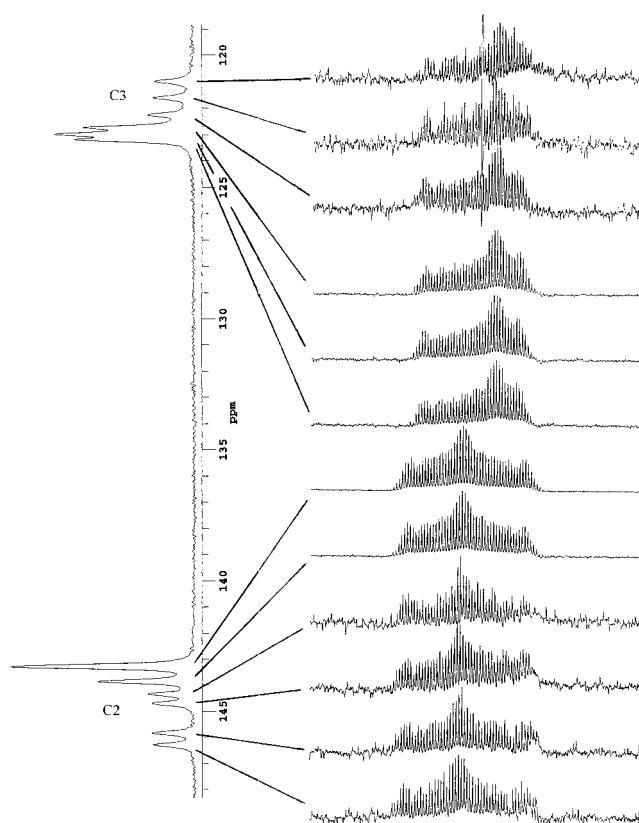


**Figure 2.** A comparison of the isotropic  $^{13}\text{C}$  spectrum before (bottom) and after (top) thermal annealing. The top spectrum was produced by both slow and rapid cooling of the melt as well as drying from a variety of solvents. Peaks between 140 and 150 ppm arise from C2 while those near 120 ppm are due to C3.

12 lines for the two carbons present (see Figure 2). For a given carbon, the additional lines were due to different conformational or crystallographic environments. This is shown by acquiring multiple  $^{13}\text{C}$  NMR isotropic spectra while varying the  $^1\text{H}$  to  $^{13}\text{C}$  cross polarization (CP) times. Nearly identical CP rates were found within sets of closely grouped lines, while lines from different spectral regions exhibited their own characteristic CP rates. Further evidence that a given set of lines originated from the same molecular position was found by noting the remarkably similar chemical shift principal values measured for the tensor patterns in any given spectral region with similar CP rates. Figure 3 exhibits the 12 individual FIREMAT tensor patterns for the 2  $\text{sp}^2$  carbons, C2 and C3.

The narrow isotropic lines support the view that verbenol is composed of a mixture of different crystalline forms rather than an amorphous mixture. This position is further strengthened by the X-ray powder analysis which shows that the powder diffracts strongly. The melting behavior of verbenol also supports this conclusion. The solid partially melts at 42–44 °C but some solid particles remain in the melt. At 59–62 °C the remaining solid melts consistent with the hypothesis of mixed crystals, each having a characteristic melting point. Finally, the X-ray structure of the more stable major form of verbenol (corresponding to the higher melting solid<sup>16</sup>) reveals three molecules per asymmetric unit. The isotropic NMR spectrum of this more stable form exhibits up to three isotropic peaks per carbon type accounting for five of the 12 observed  $\text{sp}^2$  isotropic lines in the mixed material. The presence of isotropic lines in addition to those from the major form verifies the hypothesis of a mixed crystalline solid.

We wish to comment on one possible explanation for the mixed crystalline nature of verbenol. This involves the so-called “flip-flop hydrogen bonding” hypothesis<sup>17</sup> that occurs when adjacent hydrogen bonds in concatenated molecules in the lattice switch their bonding partners. This exchange is detected in X-ray analysis as a 0.5/0.5 fractional occupancy in hydroxy hydrogens between oxygen atoms and is found in ice and some hydrocar-



**Figure 3.** Spinning sideband patterns for C2 and C3 showing the similarity of tensor patterns obtained for the individual isotropic lines of a given carbon. The six distinct lines for each carbon arise from only minor conformational variations in the solid-state molecules.

bon alcohols.<sup>18</sup> This phenomenon would lead to a mixture of conformations in the solid as each time the bonded hydrogen rotates, the hydroxy group would have a new orientation relative to the remainder of the molecule thus potentially changing the NMR shifts of nearby carbons. To test this hypothesis, a NMR exchange experiment<sup>19</sup> was performed to determine if hydroxy hydrogens reorient in verbenol. With a mixing time of 0.10 s no exchange was observed, supporting the view that verbenol consists of distinct nonexchanging (or very slowly exchanging) crystalline domains.

The commercial verbenol sample was demonstrated to be a metastable form by the spectral changes accompanying melting and resolidifying. The 12 peaks of the  $\text{sp}^2$  carbons (see Figures 2 and 3) become 5 peaks (2 for carbon 2 and 3 for carbon 3) upon either melting or dissolving then resolidifying the sample. The X-ray analysis reported was performed on a crystal obtained from this more stable form of verbenol. The difference in the two spectra identifies the lines at 146.3, 145.9, 144.8, and 144.4 ppm for carbon 2 and 122.3, 121.7, and 121.0 ppm for carbon 3 as arising from the minor polymorph. Further, examination of verbenol samples from two different sources reveals that all the minor peaks have a 1:1 ratio to each other and all decrease in the same proportion when variations are observed between samples resulting from different degrees of annealing. These results support the position that the small peaks arise from a single polymorphic form. A differential loss in peak intensity

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would likely be observed if the minor peaks represented more than one form.

NMR analyses were performed separately on both the more stable crystalline solid, for which X-ray structure is known, and the peaks of the metastable minor form found in the mixture. Separate analysis of the stable major form is desirable as NMR parameters derived from such analysis are free of the influence of the additional overlapping peaks arising from the minor form. In addition, NMR structural conclusions regarding the more stable form can be verified from the crystal structure. This provides a measure of the accuracy of our approach that becomes invaluable in predicting possible structures of the less stable form. Having determined that verbenol is a metastable mixture of two crystalline polymorphs, the molecular origins of such a mixture were investigated using chemical shift tensors.

**Structural Predictions from Computed and Experimental NMR Shift Tensors.** The measurable chemical shift tensor is a symmetric second rank tensor with six potentially observable values.<sup>20</sup> The antisymmetric portion is a higher order component that cannot be obtained from Zeeman splitting. To observe experimentally all six symmetric tensor components, single crystal data are usually required. For powdered solids the three values of the diagonalized tensor can be determined. These three values are referred to as principal values and denoted by  $\delta_{11}$ ,  $\delta_{22}$ , and  $\delta_{33}$  in order of decreasing frequency. Such tensor quantities have been demonstrated to be sensitive indicators of molecular geometry.<sup>8,9</sup>

The relatively new 2D FIREMAT experiment<sup>11</sup> has proven to be a powerful method for obtaining principal values in powders.<sup>9</sup> This experiment provides spinning sideband patterns (see Figure 3) from which principal values are derived for each carbon. These patterns are separated from one another in the second dimension by the differences in their  $^{13}\text{C}$  isotropic shifts. As originally developed, magic angle turning (MAT) experiments were hindered by long analysis times.<sup>9</sup> The FIREMAT analysis is especially well suited for rapid analysis as it uses a time domain data replication technique that further reduces spectrometer time. The replication feature of FIREMAT and the enhancement from the recent TIGER analysis, used to process the evolution dimension (isotropic shift in the case of FIREMAT), has significantly enhanced the S/N and reduced the analysis time.<sup>9,13</sup>

In verbenol the  $\text{sp}^2$  carbons exhibit extremely wide powder patterns ranging to widths of over 200 ppm. The wide patterns have associated low S/N preventing accurate analysis and historically have required prohibitively long analysis times. A corresponding FIREMAT spectrum may be acquired in only a fraction of the time yet, because of the relatively large number of spinning sidebands observed, provides remarkably high quality shift tensor data compared with other methods for studying  $\text{sp}^2$  carbons.<sup>9</sup>

Assignments of shifts to molecular positions, required for structural refinement, were made using the previously described comparison of experimental and computed tensor values.<sup>9,15</sup> All assignments to molecular position are shown in Table 1 and are statistically preferred to any other assignment at the 95% confidence level. It is impossible, however, to distinguish between congruent molecules in the asymmetric unit as discussed below. Corresponding solution  $^{13}\text{C}$  shifts determined from 2D INADEQUATE are included in Table 2 for comparison.

To rationalize the splitting in the isotropic peaks for a given carbon to specific molecular conformations, computer modeling

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**Table 1.** Verbenol Principal Values of the Major Crystalline Form, Experiment (Theory)<sup>a</sup>

C no.	$\delta_{11}$	$\delta_{22}$	$\delta_{33}$	isotropic <sup>b</sup>
1	63.6 (60.6)	43.3 (41.7)	38.7 (38.4)	48.5 (46.9)
	63.1 (61.0)	43.9 (43.9)	38.1 (37.3)	48.4 (47.4)
	63.1 (60.9)	43.7 (41.5)	37.8 (37.4)	48.2 (46.6)
2 <sup>c</sup>	248.1 (251.4)	147.9 (165.5)	35.7 (36.4)	143.9 (151.1)
	247.3 (247.7)	147.4 (165.6)	35.3 (37.4)	143.3 (150.2)
	247.3 (252.1)	147.4 (164.6)	35.3 (37.1)	143.3 (151.1)
3	224.8 (225.0)	97.0 (98.5)	47.9 (49.7)	123.2 (124.4)
	225.0 (223.6)	97.7 (99.2)	46.4 (49.5)	123.0 (124.1)
	224.0 (219.1)	98.2 (96.7)	46.1 (48.6)	122.8 (121.5)
4	97.6 (96.0)	82.6 (82.5)	39.5 (38.4)	73.2 (72.3)
	97.7 (99.2)	82.1 (82.0)	39.1 (39.1)	73.0 (73.5)
	97.3 (96.6)	81.7 (81.3)	39.3 (38.4)	72.8 (72.1)
5 <sup>c</sup>	57.1 (58.1)	46.4 (43.0)	38.2 (38.2)	47.3 (46.5)
	57.1 (58.4)	46.4 (42.2)	38.2 (36.7)	47.3 (45.8)
	56.4 (58.1)	45.6 (41.0)	37.9 (36.5)	46.6 (45.2)
6 <sup>c</sup>	59.2 (61.2)	39.7 (42.3)	23.0 (21.4)	40.7 (41.7)
	59.2 (62.1)	39.7 (42.5)	23.0 (22.6)	40.7 (42.4)
	58.5 (60.6)	39.9 (42.7)	22.1 (21.2)	40.2 (41.5)
7	53.0 (53.0)	32.9 (30.0)	22.8 (24.0)	36.2 (35.7)
	52.3 (52.3)	31.3 (30.2)	23.3 (24.1)	35.7 (35.6)
	50.9 (52.7)	29.5 (30.3)	24.3 (24.9)	34.9 (36.0)
8 <sup>c</sup>	40.8 (44.2)	30.0 (33.3)	3.5 (−0.2)	24.8 (25.8)
	40.8 (44.4)	30.0 (33.3)	3.5 (−0.2)	24.8 (25.8)
	39.9 (43.3)	30.2 (33.4)	3.2 (−0.1)	24.5 (25.5)
9	37.3 (37.4)	30.2 (31.4)	5.3 (3.9)	24.2 (24.2)
	37.1 (37.0)	29.8 (31.4)	5.0 (3.8)	24.0 (24.1)
	35.9 (36.5)	29.9 (30.9)	4.5 (3.0)	23.4 (23.5)
10	43.5 (45.3)	38.5 (39.0)	3.9 (−0.1)	28.6 (28.1)
	42.2 (44.8)	37.9 (39.2)	3.9 (−0.2)	28.0 (27.9)
	43.0 (44.9)	37.6 (38.8)	2.2 (−0.2)	27.6 (27.8)

<sup>a</sup> Theoretical values for a given carbon position are interchangeable as the various permutations with experimental values are statistically indistinguishable. <sup>b</sup> Experimental isotropic shifts were obtained from a separate CP/MAS analysis. <sup>c</sup> These carbons exhibit only two isotropic resonances per three carbons of the asymmetric unit, thus averaged principal values are obtained for isotropically degenerate lines.

**Table 2.** Solution INADEQUATE  $^{13}\text{C}$  Shifts

C no.	isotropic	$^1J_{\text{CC}}$ (Hz) <sup>a</sup>	C no.	isotropic	$^1J_{\text{CC}}$ (Hz) <sup>a</sup>
1	47.71	$J_{1,2} = 39.8$	6	38.87	$J_{6,1} = 28.1$
2	146.66	$J_{2,3} = 66.7$	7	35.34	$J_{7,1} = 27.0$
3	119.50	$J_{3,4} = 44.1$	8	22.52	$J_{8,2} = 43.2$
4	73.00	$J_{4,5} = 37.0$	9	22.62	$J_{9,6} = 41.2$
5	48.05	$J_{5,6} = 28.8$	10	26.88	$J_{10,6} = 37.3$
		$J_{5,7} = 26.6$			

<sup>a</sup> The 1 bond  $^{13}\text{C} - ^{13}\text{C}$  scalar couplings used to interpret the INADEQUATE are included here for reference.

was performed. Potentially correct geometries were prepared and the corresponding tensors computed for comparison. Verbenol has a fairly rigid bicyclic structure, thus the only significant conformational variation in the structure is associated with the dihedral angle of the hydroxy hydrogen. Twelve representative structures differing in dihedral angle were prepared and the corresponding shift tensors computed. The hydroxy hydrogen was initially oriented eclipsing the C3–C4 bond and the subsequent models were made by rotating the hydrogen in 30° increments in a counterclockwise direction for a Newman projection in which the OH bond is first placed in front of the C3–C4 bond. At each angle, the selected C3–C4–O–H dihedral angle was frozen and the remaining structure allowed to relax via an energy-minimization procedure. Chemical shift tensors were then computed for all carbons in these optimized structures. Allowing conformational variation only at the hydroxy hydrogen is supported by the X-ray data. The X-ray C3–C4–O–H dihedral angles of 55°, 69°, and 74° were the largest structural variations within the three molecules of

the asymmetric unit. All other heavy atom dihedral angles differ by  $<1^\circ$  except for two positions where differences of ca.  $2^\circ$  are found.

Previous work has demonstrated that molecules which strongly interact with their surroundings must include portions of the neighboring lattice to yield accurate computed tensors. Inclusion of such effects in the salt cadmium acetate produced overall improvements in  $^{13}\text{C}$  tensors of roughly 60 ppm.<sup>21</sup> Large improvements of 30–40 ppm were also observed in the  $^{15}\text{N}$  computed tensor of benzamide.<sup>22</sup> Recent work in our laboratory suggests that such lattice detail may also improve computed tensors in ketones and other carbonyl containing compounds when such groups act as hydrogen bond acceptors. Surprisingly, in the case of verbenol, inclusion of methanol molecules in positions occupied by neighboring molecules made only small changes in computed tensors of less than the anticipated error. Hence, inclusion of lattice detail was omitted in all computations described herein. Such neglect appears justified in the case of hydroxyl groups as previous  $^{13}\text{C}$  single crystal studies of carbohydrates have accurately computed  $^{13}\text{C}$  shift tensors using only isolated molecules.<sup>23</sup> Therefore, in verbenol, conformational effects appear to dominate tensor variation.

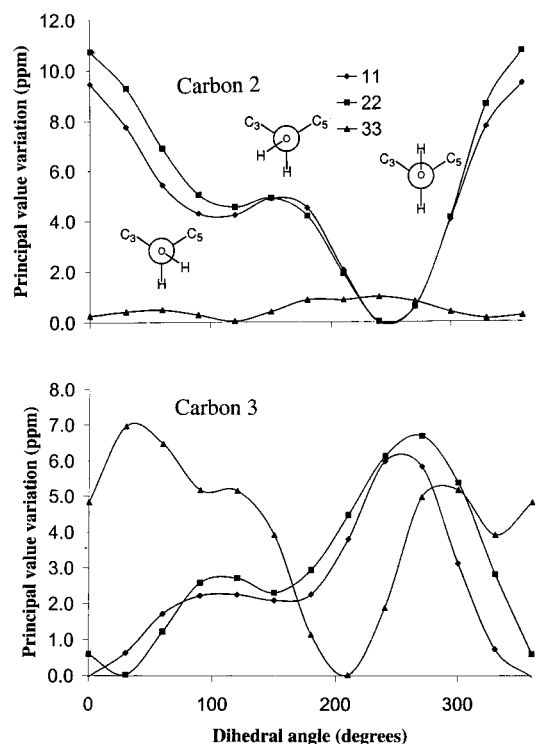
For structural analysis to be relevant, variation in the computed tensor for a given carbon associated with different geometries must exceed the computational errors. Of the carbons influenced by the hydroxy hydrogen rotation, only carbons 2, 3, 4, and 5 exhibit theoretical variations in one or more principal values that are significantly larger than the anticipated errors of about 3–4 ppm for  $\text{sp}^2$  carbons and 2 ppm for  $\text{sp}^3$  carbons.<sup>9,15</sup> Hence, only four carbons were analyzed to establish molecular conformations. Representative variations in principal values as a function of  $\text{C}3\text{--C}4\text{--O--H}$  dihedral angle are shown in Figure 4 for C2 and C3.

**Steric Restrictions on Dihedral Angle.** Having chosen reasonable model structures and established molecular positions likely to yield conformational information, it is interesting to identify probable  $\text{C}3\text{--C}4\text{--O--H}$  dihedral angles which could give rise to the observed spectra. Correct structures must have favorable van der Waals intramolecular interactions. The 12 energy minimized verbenol structures were thus examined to characterize any energetically unfavorable steric interactions with the hydroxy hydrogen. Figure 5 shows the intramolecular van der Waals energy versus dihedral angle. From this plot it is clear that orientations between  $240^\circ$  and  $360^\circ$  are highly improbable due to sterically unfavorable interactions of the hydroxy hydrogen with the methyl hydrogens of carbon 9. A computation of Boltzmann populations shows that less than 3% of the population would occupy the maxima at  $270^\circ$  relative to either of the two local minima at approximately  $60^\circ$  or  $180^\circ$ . The minima at  $60^\circ$  and  $180^\circ$  are very similar in energy and are calculated to have roughly equal populations. Orientations ranging from  $0^\circ$  to  $240^\circ$  all have energy values within 1.5 standard deviation ( $\sigma$ ) of the lowest minima and thus cannot be excluded from consideration on the basis of steric effects. However, structures proposed from shift tensor data provide additional constraints and both sets of constraints must obtain in identifying the most favorable structures.

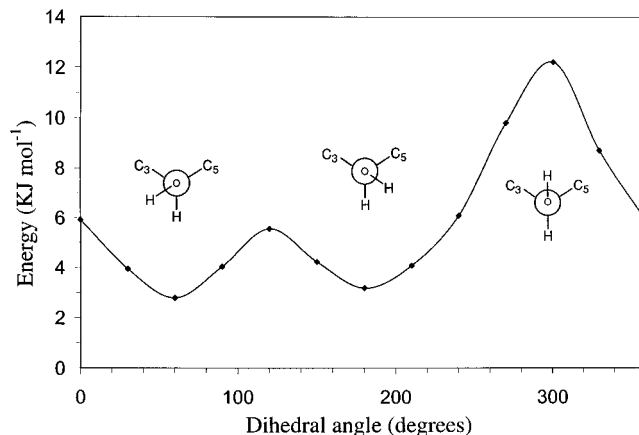
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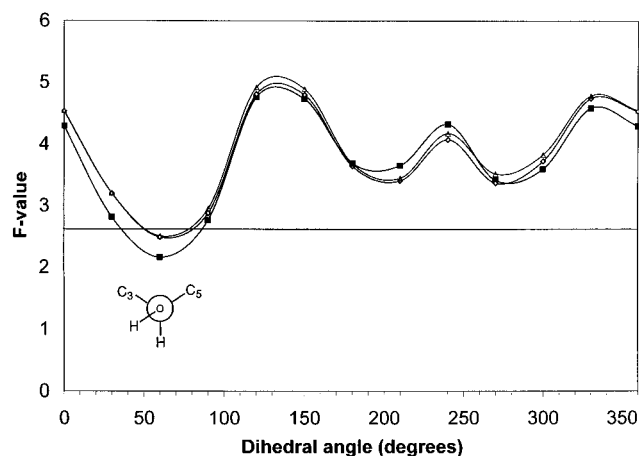
**Figure 4.** Variations in the computed tensor principal values as a function of  $\text{C}3\text{--C}4\text{--O--H}$  dihedral angle for carbons 2 and 3. Newman projections for  $60^\circ$ ,  $180^\circ$ , and  $270^\circ$  (from left to right) are included to show the direction of rotation. Similar sensitivities to conformation were observed in the tensor components of carbons 4 and 5 but are not shown. All other carbons show variations of less than the computational error and thus contribute no conformational information.



**Figure 5.** Intramolecular steric van der Waals energy associated with the rotation of the hydroxy hydrogen around the  $\text{C--O}$  axis. Unfavorable conformations between  $240^\circ$  and  $360^\circ$  arise from interactions between the hydroxy hydrogen and the protons on C9.

**Conformational Analysis of Crystalline Verbenol.** Tensor principal values can also be examined to further establish reasonable structures by comparison with model structures. Tensor data are available for each individual isotropic resonance, thus shift tensors contain peak specific information unlike the van der Waals approach. An  $F$ -test was chosen for comparing alternative conformationally varied models with experiment. The shift tensor analysis of the major polymorph of verbenol is discussed first as proposed structures can be verified from X-ray geometry. Modeling of the minor form is then considered.

Analysis of tensor values with an  $F$ -test requires comparison of a variance for the known geometry with the variances



**Figure 6.**  $F$ -value versus dihedral angle for potential model structures. Tensors were computed at  $30^\circ$  intervals in the hydroxy dihedral angle ( $\text{C3-C4-O-H}$ ) with extrapolated values used for intermediate angles. The 90% statistical confidence level is shown. In the major form, the three molecules of the asymmetric unit produce three isotropic lines for most carbons (see Table 1). Hence, each curve shown corresponds to one of the three molecules of the asymmetric unit. Assignment of a given curve to a specific molecule of the asymmetric unit was not possible using current methods.

computed for the various proposed geometries. However, computing a variance for the known verbenol geometry is complicated slightly by a statistical inability to associate clearly the three experimental peaks for a given carbon with a specific molecule in the asymmetric unit. This result suggests that the three molecules of the asymmetric unit have only minor differences in their dihedral angles of less than the error in the theory. Nevertheless, the smaller relative experimental errors still leave most of the experimental peaks clearly resolved.

For the known geometry, all permutations of theory and experiment in the asymmetric unit for a given carbon were statistically indistinguishable. It is therefore sufficient to compute a variance which consists of experimental C2, C3, C4, and C5 tensors arbitrarily paired with computed C2, C3, C4, and C5 values from the three molecules in the asymmetric unit. The uncertainties in these pairings in every case are much less than the theoretical errors in computed principal values. It should be noted that this approach implies no ambiguity in carbon position assignment, only in the assignment of a given position to one of the three unique molecules of the asymmetric unit.

Using the proposed approach, an  $F$ -value was computed at each dihedral angle.  $F$ -values were computed both for the C2, C3, C4, and C5 individual positions and with all four carbons combined. As might be anticipated, the individual values yield less information than the combined data, with C4 providing the maximum structural detail. Specifically, data from individual positions alone eliminate dihedral angles ranging from  $110^\circ$  to  $160^\circ$ . In contrast, a comparison which combines principal values from all four carbons (excluding the  $\delta_{22}$  component of C2 that contains a large systematic computational error) eliminates all but a range of approximately  $\pm 20^\circ$  as shown in Figure 6. The correct dihedral angles of  $55^\circ$ ,  $69^\circ$ , and  $74^\circ$  found in the major form are included in the allowed regions thus validating the approach chosen. Unfortunately, the inability to differentiate the different molecules of the asymmetric unit by NMR prevents associating a specific curve with one of the specific molecules of the asymmetric unit.

Analogous structural predictions can be made using isotropic shifts alone. Such values are more commonly available, but are usually less informative as the number of parameters for

**Table 3.** Experimental Principal Values for the Minor Form of Verbenol

C no.	$\delta_{11}$	$\delta_{22}$	$\delta_{33}$	isotropic
2	250.7	151.7	36.5	146.3
2	251.9	151.0	34.7	145.9
2	250.2	149.8	34.3	144.8
2	249.1	148.3	35.7	144.4
3	224.2	96.5	46.2	122.3
3	218.9	99.1	47.0	121.7
3	226.6	93.2	43.4	121.0
9	39.1	27.4	1.4	22.6
10	41.1	36.2	3.9	27.1
10	42.2	34.9	3.0	26.7

comparison is less and the averaging required to obtain such shifts allows for error cancellation. Experimental isotropic shifts were compared to the various computed shifts using a  $t$ -test. Carbons 4 and 5 yielded no information with all modeled angles statistically indistinguishable from the experimental shifts. Carbon 3 supports the van der Waal conclusions by eliminating dihedral angles between  $250^\circ$  and  $280^\circ$  as possible solutions. The carbon 2 shift values are of less value due to the previously described systematic error in the computed  $\delta_{22}$  value which must be included to create the isotropic value. Hence, principal values provide a significantly more accurate determination of dihedral angle than is obtainable from isotropic shifts alone.

**Characterization of the Minor Polymorphic Form of Verbenol.** Having established and verified an approach for predicting structures for crystalline verbenol, the minor form is now treated. This form, while differing from the major form, was available only as a crystalline mixture containing just 10 isotropic lines that are distinctly resolved from those in the major form. Four of these lines arise from C2, three from C3, two from C10, and one from C9. Table 3 lists all measured principal shift values for the additional 10 unique peaks. All remaining peaks observed arise primarily from the major form and measured principal values for these peaks are within the anticipated experimental error of those reported in Table 1. Hence, principal values for peaks common to both forms are omitted from the analysis of the mixed material. Examination of computed tensors shows that only C2 and C3 exhibit variations in tensor components as a function of the hydroxy orientation that are greater than the anticipated errors. Hence, these carbons were used to evaluate feasible dihedral ranges. All analyses were performed on C2 and C3 values separately as these carbons have different numbers of isotropic peaks making combining impossible.

The  $F$ -test for carbon 2 alone indicates that all dihedral angles are statistically indistinguishable from each other. Analysis of carbon 3 eliminated dihedral ranges of  $200-320^\circ$ ,  $230-280^\circ$ , and  $250-280^\circ$  as possible solutions for the peaks at 122.3, 121.0, and 121.7 ppm, respectively. Thus, for this minor form, tensor data add only marginally to the information already available from the van der Waals restrictions. Fortunately, other structural conclusions can still be made regarding this form from the NMR data.

The number of isotropic lines and relative peak ratios for the minor form infer important lattice details. The constant relative ratios of the 10 minor isotropic peaks, regardless of the source or preparation method, are evidence that these peaks arise from a single polymorph. Further, the presence of four distinct lines of the same intensity for C2 indicates a lattice consisting of four molecules per asymmetric unit.

Potentially valid dihedral angles for the minor form are suggested from the van der Waal plot and the isotropic spectrum.

Two of C2's minor peaks are nearly isotropically degenerate with those of the major form, while the remaining two are separated by nearly 2 ppm from the nearest line of the major form. These results suggest two molecules with dihedral angles similar to those in the major form and two with different angles. Carbon 3 strengthens this conclusion with two peaks well separated and a second pair consisting of only one barely resolved line with the second degenerate with the center peak from the major form as indicated by intensity differences in this peak in Figure 2. The minima in the van der Waals curve near  $60^\circ$  and  $180^\circ$  suggest a tetramer consisting of molecules with dihedral angles near  $60^\circ$  and  $180^\circ$  but more information is required to complete the analysis.

A previous survey of lattice structures found in similar hydrocarbon alcohols has found that ring, chain, and helical structures are common.<sup>24</sup> Hence, for the minor form, no lattice arrangement can be confirmed from this initial investigation. However, this analysis demonstrates the ability of NMR data to provide information on the number of polymorphs present, the number of molecules per asymmetric unit in mixed polymorphic materials, and ranges of dihedral angles potentially present. For materials for which no crystals are available, such information has traditionally been difficult to obtain.

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## Conclusions

Previously detailed analysis of the composition of mixed crystalline solids has been difficult or impossible. This work on verbenol demonstrates that new solid-state NMR methods, such as FIREMAT and TIGER, may allow rapid and accurate analysis of such materials. These analyses allow the number of lattice forms to be identified by the number of isotropic lines of the same intensity. Further, comparison of tensor principal values with computed values for model structures extends the analysis to provide a reasonable estimation of molecular conformation. These techniques are, perhaps, most relevant to analysis of pharmaceutical materials where both the lattice composition of solids and presence of polymorphic mixtures capable of modifying bioactivity can be easily determined regardless of their tendency to form crystals suitable for X-ray analysis. The feasibility of correlating H—O—C—C dihedral angles with chemical shift tensors has been discussed, and the method is sufficiently general that extension to molecules with greater conformational variety appears promising.

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