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# Complete Spectral Analysis of the $^1$H NMR 16-Spin System of β-Pinene

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**The complete analysis of the $^1$H NMR spectrum of β-pinene, (1*S*)-(−)-6,6-dimethyl-2-methylenebicyclo[3.1.1]heptane, which is of the ABCDEFGHIJX$_3$Y$_3$ type, is reported and earlier results are corrected. The vicinal coupling constants, $^3J$(H,H), are compared with the theoretical values calculated by using the Altona and co-workers' equations for the structure derived by molecular modelling. The results were applied to the conformational analysis of β-pinene.** © **1997 by John Wiley & Sons, Ltd.**

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## INTRODUCTION

The analysis of the 1D $^1$H NMR spectra of large spin systems of alicyclic compounds such as terpenoids, often characterized by a large number of long-range couplings, is a challenge for computerized spectral analysis.

β-Pinene, (1*S*)-(−)-6,6-dimethyl-2-methylenebicyclo [3.1.1]heptane, possessing a variety of different structural fragments, also serves as a useful model system to test relationships between structure and NMR parameters. Therefore, it is important that the analytical methods used are able to determine all NMR parameters of β-pinene as accurately as possible.

Smith[1] reported a re-examination of the $^1$H NMR spectrum of β-pinene by two-dimensional NMR methods at 300 MHz and by using lanthanide shift reagents. The study was undertaken owing to the observed discrepancies between the spin–spin coupling constants in two previous papers.[2,3] However, Smith reported neither a computer-assisted $^1$H NMR spectral analysis nor a full set of NMR parameters for β-pinene, although these values are of great importance regarding the structural and conformational properties of the molecule. An accurate determination of the conformationally informative vicinal coupling constants, however, requires a complete spectral analysis since the majority of the signals show very complex splitting patterns owing to the presence of many long-range couplings. Therefore, the availability of a 400 MHz high-resolution spectrum of β-pinene and a novel NMR spectral analysis procedure[4,5] prompted us to attempt once more the analysis of this naturally occuring monoterpene.

## RESULTS AND DISCUSSION

The structure and numbering of β-pinene are described in Fig. 1. In contrast to the notation of Smith[1] we use the conventional one where the methyl group *syn* to the exocyclic methylene is numbered 9, in agreement with Abraham *et al.*[6] The $^1$H NMR chemical shifts and spin–spin coupling constants are given in Tables 1 and 2. Table 2 also contains the values given by Abraham *et al.*[6] and the vicinal coupling constants calculated by the equations of Altona and co-workers[7] based on the PM3[8] optimized structure of β-pinene. Partial $^1$H NMR spectra (calculated and experimental) of β-pinene are illustrated in Figs 2–4.

The present results are in agreement with the NMR parameters reported by Abraham *et al.*[6] in 1973, which

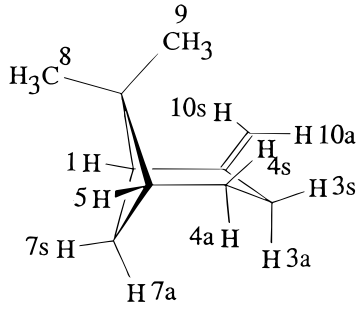


**Figure 1.** Schematic structure and numbering of β-pinene.

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**Table 1. $^1$H NMR chemical shifts of β-pinene at 400 MHz (δ, ppm, from TMS)[a]**

| Proton | | δ($^1$H) | |
|---|---|---|---|
| H-10a | 4.601[b] | 4.767[c] | 4.57[d] |
| H-10s | 4.538 | 4.731 | 4.50 |
| H-3a | 2.542 | 2.515 | 2.52 |
| H-1 | 2.441 | 2.514 | 2.43 |
| H-7s | 2.338 | 2.281 | 2.31 |
| H-3s | 2.245 | 2.213 | 2.23 |
| H-5 | 1.973 | 1.914 | 1.97 |
| H-4s | 1.857 | 1.857 | 1.85 |
| H-4a | 1.830 | 1.811 | 1.82 |
| H-7a | 1.419 | 1.418 | 1.42 |
| 8-Me | 1.233 | — | 1.24 |
| 9-Me | 0.719 | — | 0.73 |

[a] The standard errors given by the computer analysis were *ca*. 0.002 Hz.
[b] In $(CD_3)_2CO$.
[c] In $C_6D_6$.
[d] In $CCl_4$, by Abraham *et al*.[6]

were, unfortunately, overlooked by later workers.[1–3] The average difference in the absolute values of the coupling constants is 0.36 Hz. This deviation represents the bias caused by an inability to take into account all long-range coupling constants, which is possible only by a full computerized analysis of the spectrum. Based on our previous COLOC experiments[2] and the present spectral analysis, the assignment of the exocyclic methylene protons (H-10a and H-10s) given in Refs 2 and 3 and adopted by Smith[1] should be interchanged. Similarly, the assignment of methyls 8 and 9 given by Smith[1] should be reversed, as proved by us using a lanthanide shift reagent for pinocarvonce[2] and by Wenzel and Sievers[9] for β-pinene itself. Furthermore, the assignment of H-3a and H-3s in Refs 2 and 3 is now reversed and in accord with that given in Refs 1 and 6.

## Spectral analysis

The spectral analyses were performed with the PERCHit iterator[5] under PERCH software[4] using a Pentium 150 MHz personal computer. A typical simulation time of the ABCDEFGHIJX$_3$ spectrum (one of the methyls was decoupled) was 30 s. The correct solution was found by using the integral-transform mode, while the final refinement of the result was performed by using the total lineshape fitting option of the PERCHit iterator. Some details of the strong resolution enhanced spectrum of the H-10a (Fig. 3) in acetone-$d_6$ demanded a 'manual,' non-iterative refinement of the smallest coupling constants.

The signals of H-1, H-7a and H-10a constituted a special problem: very strong windowing was required before any fine structure of the signals was visible from the methyl decoupled spectra. The strong windowing led to noisy signals of the other protons. The analysis of the parameters of H-1, H-7a and H-10a was performed based on a spectrum combined from two partial spectra obtained by different resolution enhancements. The spectrum in benzene-$d_6$ was much closer to the first-order spectrum than in acetone-$d_6$. Therefore, the spectrum in benzene was very helpful in finding good trial values for the coupling constants of the H-10a (Fig. 3). On the other hand, the changes of the coupling constants and the second-order effects led to non-resolved fine structure in the signals of H-1 and H-7a. The signal of H-7a was poorly resolved in both solvents. Its long-range couplings are based on the information for the other signals of β-pinene.

The spectral analysis was performed in two stages. The spectrum with the 8-CH$_3$ protons (less shielded) decoupled was analysed at first. The couplings of the 8-CH$_3$ protons were then obtained from the 9-CH$_3$ decoupled spectrum by keeping the remaining couplings fixed during the iteration. The trial values for the methyl couplings were also based on the decoupling

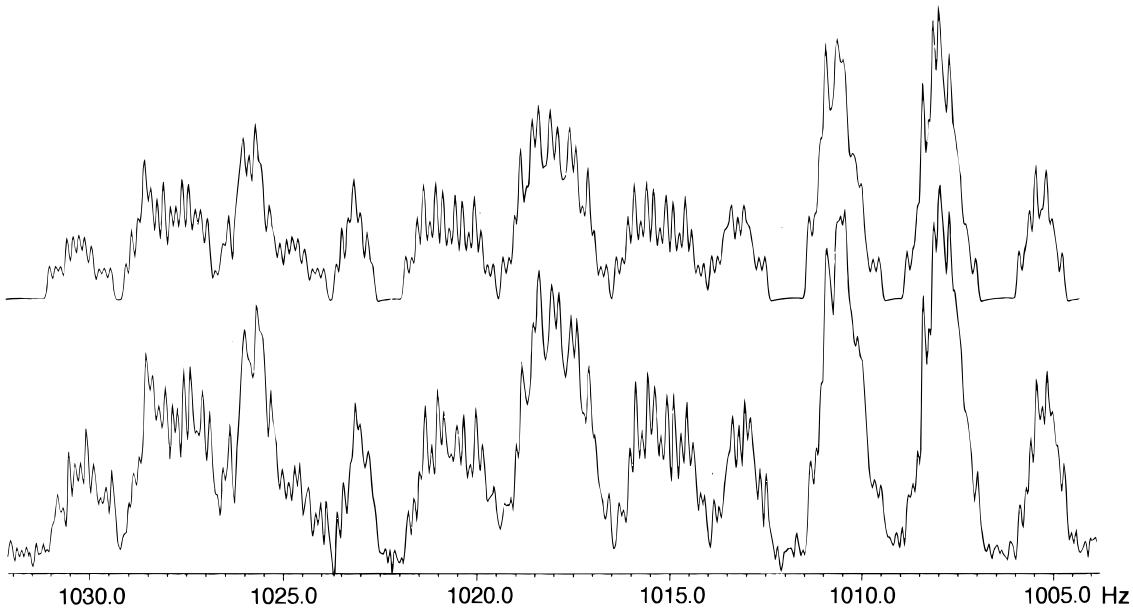


**Figure 2.** Part of the $^1$H NMR signal of the proton 3a at 400 MHz: experimental spectrum (bottom) and calculated spectrum (top).

**Table 2. $^nJ$(H,H) coupling constants (Hz) of β-pinene**

| Protons | n | $^nJ$(H,H)[a] Experimental | | | Calculated[e] |
|---|---|---|---|---|---|
| 10a, 10s | 2 | **+2.09**[b] | 1.99[c] | 1.5[d] | — |
| 10a, 3a | 4 | **−2.53** | −2.51 | 2.5 | — |
| 10a, 1 | 4 | **−0.09** | −0.14 | — | — |
| 10a, 7s | 5 | +0.31 | 0.30 | — | — |
| 10a, 3s | 4 | **−1.26** | −1.27 | 1.3 | — |
| 10a, 5 | 6 | −0.20 | −0.20 | — | — |
| 10a, 4s | 5 | **+0.05** | 0.03 | — | — |
| 10a, 4a | 5 | **+0.05** | 0.04 | — | — |
| 10a, 7a | 5 | +0.10 | 0.12 | — | — |
| 10a, 8 | 6 | 0.00 | ? | — | — |
| 10a, 9 | 6 | 0.00 | 0.00 | — | — |
| 10s, 3a | 4 | **−2.96** | −2.93 | 2.5 | — |
| 10s, 1 | 4 | **−0.54** | −0.55 | — | — |
| 10s, 7s | 5 | **−0.05** | −0.06 | — | — |
| 10s, 3s | 4 | **−1.15** | −1.17 | 1.3 | — |
| 10s, 5 | 6 | −0.03 | −0.03 | — | — |
| 10s, 4s | 5 | **−0.03** | −0.03 | — | — |
| 10s, 4a | 5 | **+0.51** | 0.50 | — | — |
| 10s, 7a | 5 | −0.03 | −0.07 | — | — |
| 10s, 8 | 6 | 0.00 | ? | — | — |
| 10s, 9 | 6 | −0.03 | −0.01 | — | — |
| 3a, 1 | 4 | **−0.31** | −0.30 | — | — |
| 3a, 7s | 5 | **+0.51** | 0.50 | — | — |
| 3a, 3s | 2 | **−17.44** | −17.41 | −18.0 | — |
| 3a, 5 | 4 | **−0.34** | −0.33 | — | — |
| 3a, 4s | 3 | **+7.67** | 7.63 | 7.4 | 7.66/7.23 |
| 3a, 4a | 3 | **+10.79** | 10.76 | 9.7 | 11.20/10.60 |
| 3a, 7a | 5 | +0.17 | 0.16 | — | — |
| 3a, 8 | 6 | −0.04 | ? | — | — |
| 3a, 9 | 6 | −0.04 | 0.03 | — | — |
| 1, 7s | 3 | **+5.56** | 5.57 | 5.5 | 8.70/8.42 |
| 1, 3s | 4 | +0.20 | 0.18 | — | — |
| 1, 5 | 4 | **+5.32** | 5.32 | 5.0 | — |
| 1, 4s | 5 | +0.03 | 0.03 | — | — |
| 1, 4a | 5 | +0.02 | 0.06 | — | — |
| 1, 7a | 3 | **+0.37** | 0.40 | — | 1.60/0.97 |
| 1, 8 | 4 | −0.14 | ? | — | — |
| 1, 9 | 4 | −0.30 | −0.25 | — | — |
| 7s, 3s | 5 | **−0.28** | −0.27 | — | — |
| 7s, 5 | 3 | **+6.07** | 6.06 | 5.8 | 9.88/8.57 |
| 7s, 4s | 4 | **+1.53** | 1.55 | 1.5 | — |
| 7s, 4a | 4 | **−0.31** | −0.32 | — | — |
| 7s, 7a | 2 | **−9.83** | −9.84 | 10.0 | — |
| 7s, 8 | 5 | +0.05 | ? | — | — |
| 7s, 9 | 5 | **+0.05** | 0.03 | — | — |
| 3s, 5 | 4 | **+0.26** | 0.24 | — | — |
| 3s, 4s | 3 | **+9.27** | 9.30 | 8.5 | 11.17/10.61 |
| 3s, 4a | 3 | **+1.61** | 1.65 | 2.5 | 1.22/0.64 |
| 3s, 7a | 5 | +0.07 | 0.05 | — | — |
| 3s, 8 | 6 | 0.00 | ? | — | — |
| 3s, 9 | 6 | +0.05 | 0.03 | — | — |
| 5, 4s | 3 | **+1.80** | 1.82 | 2.0 | 2.00/1.93 |
| 5, 4a | 3 | **+4.44** | 4.42 | 4.0 | 4.75/4.96 |
| 5, 7a | 3 | **+0.52** | 0.53 | — | 0.92/1.10 |
| 5, 8 | 4 | −0.07 | ? | — | — |
| 5, 9 | 4 | −0.24 | −0.25 | — | — |
| 4s, 4a | 2 | **−13.30** | −13.32 | −13.5 | — |
| 4s, 7a | 4 | **−0.21** | −0.20 | — | — |
| 4s, 8 | 5 | +0.06 | ? | — | — |
| 4s, 9 | 5 | **+0.13** | 0.15 | — | — |
| 4a, 7a | 4 | **−0.27** | −0.28 | — | — |
| 4a, 8 | 5 | +0.18 | ? | — | — |
| 4a, 9 | 5 | **+0.07** | 0.09 | — | — |
| 7a, 8 | 5 | +0.10 | ? | — | — |
| 7a, 9 | 5 | 0.00 | 0.00 | — | — |
| 8, 9 | 4 | 0.00 | — | — | — |

[a] Signs of bold couplings are based on the spectral analysis, otherwise the signs are tentative.
[b] In $(CD_3)_2CO$.
[c] In $C_6D_6$.
[d] In $CCl_4$.[6]
[e] Altona and co-workers.[7]

experiments. However, it is obvious that the values of the methyl couplings that are smaller than the spectral linewidth (0.08 Hz) are not very reliable and are affected by the value given for the linewidth during the iteration.

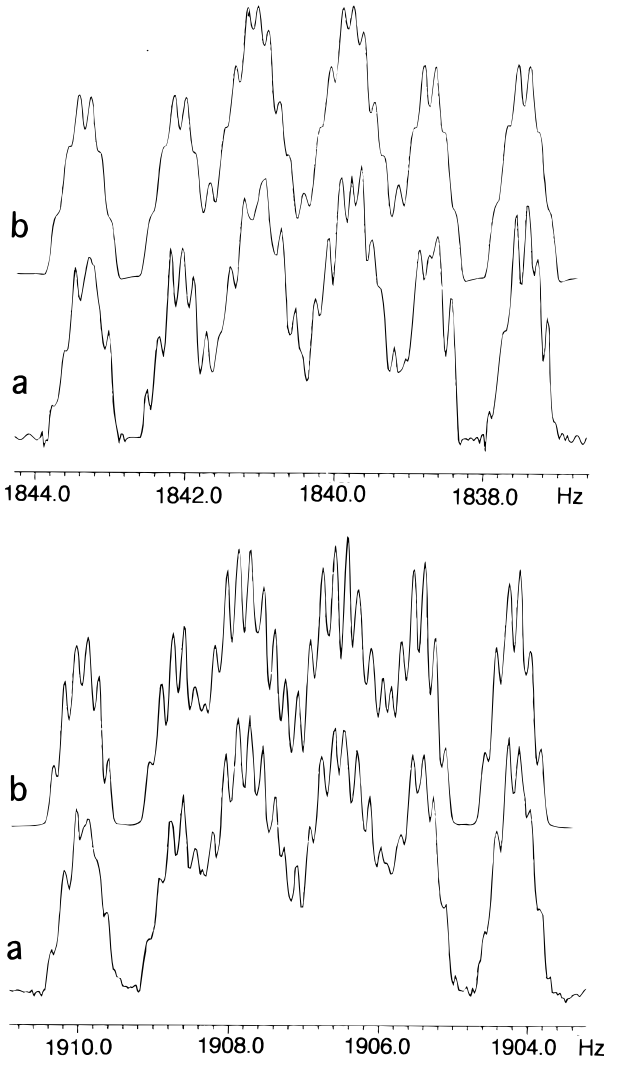


**Figure 3.** Experimental and calculated $^1$H NMR signals of proton 10a at 400 MHz in $(CD_3)_2CO$ (top) and in $C_6D_6$ (bottom). The differences between the observed (a) and computed (b) spectra can be accounted for by the strong windowing needed to resolve the fine structure. The coupling pattern of the top spectra should be symmetrical; this can be used in assessing the significance the differences between the computed and observed spectra.

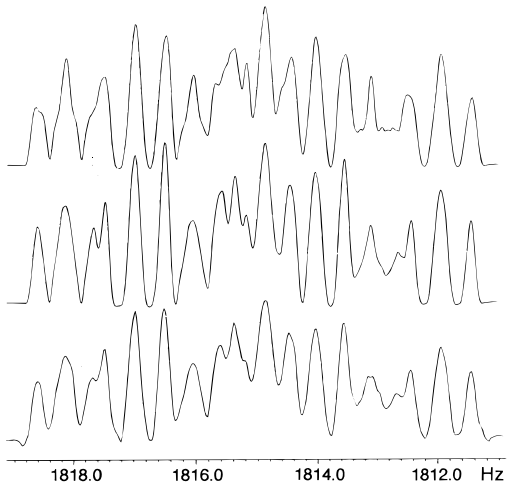


**Figure 4.** $^1$H NMR signal of the proton 10s at 400 MHz: experimental spectrum (bottom), calculated spectrum using $^5J$(10s,4s) = −0.03 Hz (middle) and calculated spectrum using $^5J$(10s,4s) = +0.03 Hz (top).

The strong geminal coupling between H-4a and H-4s made it possible to deduce signs of coupling constants. The final results are given in Table 2. All vicinal couplings and the geminal coupling within the exocyclic methylene group were set positive while all other geminal couplings were set negative. According to the model of Barfield *et al.*,[10] all allylic $^4J$ coupling constants can be assumed to be negative. The remaining signs given in Table 2 were determined by a systematic alteration of the sign combination of the couplings. In general, the signs and values of the long-range couplings follow the well known rules. The $^4J$ couplings arising from a W-pathway were the largest and normally positive, as were the $^5J$ couplings. As an example, the observed splitting patterns were reproduced precisely only with a positive value for $^5J$(10a,4s). In contrast, $^5J$(7s,3s) and also some $^4J$ couplings had to be kept negative in order to find a satisfactory solution.

The experimental vicinal $^3J$(H,H) coupling constants were related to the calculated values obtained by employing Altona and co-workers' equations[7] for the dihedral angles of the PM3[8] optimized structure of $\beta$-pinene. The calculations were done using the program VICI, which is an interactive graphical tool included in the PERCH software.[4,5] The calculated values are in qualitative agreement with the experimental values. The greatest differences are found for $^3J(1,7s) = 5.56$ Hz (calc. 8.70/8.42 Hz), $^3J(1,7a) = 0.52$ Hz (calc. 1.60/0.97 Hz), $^3J(5,7s) = 6.07$ Hz (calc. 9.88/8.57 Hz), $^3J(5,7a) = 0.52$ Hz (calc. 0.92/1.10 Hz), $^3J(3s,4a) = 1.61$ Hz (calc. 1.22/0.64 Hz) and $^3J(3s,4s) = 9.27$ (calc. 11.17/10.61). Four of them belong to the strained four-membered ring system while for the flexible six-membered ring system the results are fairly good.

## Error analysis

Owing to the total lineshape procedure used in the final refinement of the spectral parameters,[11] the accuracy of the spectral parameters can be far better than the digital resolution (0.026 Hz). On the other hand, the standard errors (varying from 0.002–0.010 Hz) provided by the standard statistical analysis are far too optimistic because the actual lineshape is unknown and also due to an extensive overlap of the spectral lines. In this particular case, the signal of one proton (which is coupled with all other protons in the molecule) consists of up to *ca.* 2000 non-degenerate transitions. Consequently, no single lines to be used for the lineshape analysis exist. Further, the minimum linewidth of 0.10 Hz obtained by the deconvolution with the program TLS[11] is almost the same as the smallest observed splittings, *ca.* 0.11 Hz. Thus, an attempt to use an underestimated linewidth can be compensated as an increase in the small couplings (particularly those of the methyls). This explains why the total lineshape fitting procedure did not lead to a good convergence of the linewidth.

In order to examine the effect of the linewidth, the latter was varied from 0.06–0.10 Hz. A Gaussian–Lorentzian lineshape (120/−20) with a dispersion contribution of 10% was used.[11] Although the rms fits (1.4% of the maximum signal in the total lineshape mode) were not sensitive to the linewidth, it was difficult to resolve the signals of the protons 7a and 10a using a linewidth larger than 0.07 Hz. In the final fitting the linewidth was set to 0.08 Hz.

A comparison of the results of the analysis in two solvents and with varying linewidths suggests that a reasonable estimate for the standard errors of all the couplings is 0.015–0.030 Hz. In Table 2 also are given some coupling values that are smaller than the linewidth. The determination of such small couplings and their signs are based substantially on the second-order effects. As an example, in Fig. 4 are shown the experimental multiplet of the proton 10s (bottom) and two simulated patterns of the same proton by using $^5J$(10s, 4s) = −0.03 Hz (middle) and $^5J$(10s,4s) = +0.03 Hz (top), respectively.

## Molecular dynamics simulation

Although $\beta$-pinene possessing a fairly strained bicyclic structure is conformationally restricted, it still exhibits some structural flexibility in the six-membered ring system as revealed by molecular dynamics simulation. The starting structure in these calculations was a PM3-optimized $\beta$-pinene.[8] The molecular dynamics simulation was done by heating the system from 0–303 K in 1 ps, keeping it 2 ps at 303 K. The data points were collected during the constant-temperature period at 303 K. All the calculations were performed using the HyperChem program package.[8]

The dihedral angles, $\theta$, between the protons at carbons 3 and 4 show remarkable asymmetric fluctuations; the ranges being (with statistical average values in parentheses). $\theta$(3a,4a) = −19.0–44.1° (16.4°), $\theta$(3a,4s) = 96.3–153.5° (132.3°), $\theta$(3s,4a) = 71.1–127.6° (100.3°) and $\theta$(3s,4s) = −13.6–37.7° (15.7°). The corresponding fluctuations in the vicinal coupling values are within the following limits: $^3J$(3a,4a) = 9–12 Hz, $^3J$(3a,4s) = 1–11 Hz, $^3J$(3s,4a) = 1–6 Hz and $^3J$(3s,4s) = 8–12 Hz, respectively. Consequently, when the dihedral angles are small, the influence of the molecular fluctuation on the vicinal coupling constant is relatively small, as in case of $^3J$(3s,4s) and $^3J$(3a,4a), whereas for $^3J$(3a,4s) and $^3J$(3s,4a) it is very large.

When the $^3J$ values are known, Altona and co-workers' equation[7] can be used inversely to calculate the dihedral angles between the vicinal CH bonds. For example, in the case of $\theta$(3s,4s), four values, viz. 31° from $^3J$(3s,4s), 22° from $^3J$(3a,4a), 15° from $^3J$(3a,4s) and 19° from $^3J$(3s,4a), respectively, are obtained. In the case of $^3J$(3s,4s) this calculation is straightforward (a graphical plot is calculated by VICI,[4,5] giving the dihedral angles directly). In other cases, the dihedral angle between the CH bonds concerned is calculated first. Then the dihedral angle, $\theta$(3s,4s), is calculated by assuming that the tetrahedral geometry for both the carbons (or 120° angles in the Newman projections) are valid. The average value of 22° for $\theta$(3s,4s) calculated in this way is in fair agreement with the optimized value (19°) and also with the expectation value given by the molecular dynamics simulation (15.7°). These results indicate that the six-membered ring system adapts a slightly chair-type conformation.

 

## CONCLUSIONS

The complete $^1$H NMR spectral analysis of $\beta$-pinene served as a critical test for the usefulness of a novel NMR analytical tool[4] and especially for the capability of the integral transform procedure[5] in such analysis. It seems that the spin system of 16 coupled protons $\beta$-pinene represents the upper limit for a system where the magnitudes of the coupling constants between all the nuclei can be analysed within acceptable confidence and by reasonable efforts. We also found that the solvent effects are very useful in finding fair trial values of the spectral parameters. Significant deviations were observed between the observed and calculated vicinal coupling constants in the four-membered ring system. This finding cannot be accounted for by the inability of molecular dynamics calculations to optimize the geometry, although the strained ring systems are known to be problematic for molecular mechanics calculations.[12] Rather, it is due to an insufficient parameterization of Altona and co-workers' equations,[7] which fail to estimate coupling constants accurately in this particular case. The most significant deviations, for $^3J$(1,7s) and $^3J$(5,7a), can be explained by the presence of an additional coupling pathway via the bridge carbon (C-6).

## EXPERIMENTAL

(1*S*)-(−)-6,6-Dimethyl-2-methylenebicyclo[3.1.1]heptane ($\beta$-pinene) was obtained from Fluka and was purified by distillation and collecting a fraction of b.p. 165–166 °C. Three $^1$H NMR spectra of a solution of $\beta$-pinene in acetone-$d_6$ (40 mg ml$^{-1}$) were recorded on a Bruker AM 400WB spectrometer operating at 400.13 MHz and 310 K: one spectrum without any decoupling and two spectra with one of the methyl signals decoupled. The data point resolution after zero-filling was 0.026 Hz. Also, a spectrum of a benzene-$d_6$ solution (100 mg ml$^{-1}$ at 320 K, with decoupling of the 8-$CH_3$ signal) was measured. The FIDs were transferred from the spectrometer to a personal computer to be handled by the PERCH software[4,5] under which the Fourier transform was performed after a strong resolution enhancement as described previously.[13] Owing to the strong windowing and broad multiplets, a careful baseline correction and an intensity calibration[11] were necessary.

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