

Construction of energy level diagram of oriented benzene using connectivity information obtained by modified Z-COSY experiment

R CHRISTY RANI GRACE¹ and ANIL KUMAR^{1,2*}

¹Department of Physics, and ²Sophisticated Instruments Facility, Indian Institute of Science, Bangalore 560012, India

Abstract. Experiments involving selective perturbation of a transition yield information about the directly connected transitions, which in turn yield information for deriving the parameters of the spin Hamiltonian of oriented molecules. Problems involved with selective perturbation are removed by the use of a two-dimensional experiment, namely, the modified Z-COSY experiment. The use of this experiment is demonstrated for obtaining the connectivity information and for determining the parameters of the spin Hamiltonian of oriented benzene, a strongly coupled six-spin system.

Keywords. Assignment of spectra; connected transitions; NMR of oriented molecules; two-dimensional (2-D) NMR.

1. Introduction

Molecules partially oriented in liquid crystalline media retain intramolecular dipolar couplings scaled by the order parameter and give rise to rich NMR spectra containing detailed information on the conformation and geometry of the molecules (Khetrapal and Diehl 1969). The scaled dipolar couplings are often large or comparable to the chemical shifts, making the spins strongly coupled. The analysis of such spectra becomes complex necessitating numerical methods for the analysis. From the observed line frequencies and line intensities one tries to obtain the various parameters of the Hamiltonian by using iterative methods. This algorithm yields a convergent result in many cases, but encounters difficulties when the number of coupled spins increases beyond 6 or 7. Any additional information about the molecule, the spin Hamiltonian and the spectrum is helpful in such an analysis. Many methods have been developed for obtaining such information. These are spin-tickling double resonance experiments (Anderson *et al* 1963; Hoffman and Forsen 1966; Freeman *et al* 1970; Anil Kumar and Gordon 1971), two-dimensional methods such as COSY (Aue *et al* 1976; Bax and Freeman 1981; Bax 1982; Griesinger *et al* 1985, 1986; Mueller 1987; Chandrasekher and Anil Kumar 1988; Ernst *et al* 1991) and Z-COSY (Oschkinat *et al* 1986; Pfändler and Bodenhausen 1991), multiple quantum spectroscopy (Drobny *et al* 1979; Bodenhausen *et al* 1980; Sinton and Pines 1980; Warren *et al* 1980; Braunschweiler *et al* 1983; Weitekamp 1983) and multiple quantum filtered correlation experiments (Piantini *et al* 1982; Rance *et al* 1983; Meier *et al* 1984). Methods involving selective perturbation of a single transition are also extremely informative (Pachler and Wessels

*For correspondence

1973; Sørensen *et al* 1974; Jakobsen *et al* 1974). For example, if a transition is selectively perturbed (say saturated or inverted), all other transitions which are directly connected to this transition are perturbed in intensity and are easily identified in a difference experiment (Grace and Anil Kumar 1992). A series of such experiments on connected transitions can reveal the entire network of connectivities. The difficulties associated with selective perturbations are removed by the use of a two-dimensional experiment (named modified Z-COSY experiment) which uses the pulse sequence $90^\circ - t_1 - \alpha - \tau_m - \beta - t_2$, where only the longitudinal magnetization is retained during the short mixing time (τ_m) and α and β are small angle pulses (Grace and Anil Kumar 1992). The small value for the angle of α pulse ensures that each cross-section parallel to ω_2 at a given ω_1 frequency is equivalent to a selective 1-D experiment in which the transition corresponding to the diagonal peak is selectively inverted at $\tau_m = 0$. Small value for the angle of the β pulse ensures that the state of the spin system is faithfully measured by this pulse. This pulse sequence is the same as a Z-COSY experiment which has been designed to monitor multispin Z orders and which uses $\alpha = \beta$ (Oschkinat *et al* 1986; Pfändler and Bodenhausen 1991). The connectivity information obtained from the modified Z-COSY experiment is converted into a connectivity matrix, which is then used in a computer program ELEV for constructing the energy level diagram. This algorithm has been demonstrated for obtaining the energy levels of oriented acetone (Grace and Anil Kumar 1992) and oriented *cis,cis*-mucanitrile (Grace *et al* 1994).

An application of this experimental algorithm to the analysis of the spectrum of oriented benzene is given in this paper. Oriented benzene has six dipolar coupled spins and forms a spin system of the type AA'A''A'''A''''A'''''. It has a symmetry of reorientation characterized by the rotational group D_{6h} and yields transitions which can be grouped into six irreducible representations namely, A_1, A_2, B_1, B_2, E_1 and E_2 . A COSY experiment separates out each of these representations in a straightforward manner by the presence of cross-peaks only between transitions of the same representation (Rukmani and Anil Kumar 1987). Multiple quantum spectroscopy has also been performed in oriented benzene and the parameters of the spin Hamiltonian have been obtained using 4 and 5 quantum spectra (Drobny 1984, 1985). Saupe (1965) has shown that the frequencies and intensities of only three transitions of this system can be given exactly and the others can be derived by making the assumptions that, (a) the dipolar couplings are in the ratios expected for a regular hexagon (figure 1), that is $D_{12} = 3 \times 3^{1/2} D_{13} = 8D_{14}$ and (b) scalar couplings J_{ij} be neglected in the off-diagonal elements of the Hamiltonian matrix. In general benzene can be characterized by six parameters: $D_{12}, D_{13}, D_{14}, J_{12}, J_{13}, J_{14}$ respectively for the direct and indirect couplings. These parameters reduce to four if assumption (a) is taken into consideration.

2. Experimental results

Figure 1 shows the 1-D spectrum of benzene oriented in ZLI-1167, along with the numbering of transitions. This show 33 pairs of transitions symmetric about the centre. Figure 2 shows the modified Z-COSY spectrum of benzene in ZLI-1167 for $\alpha = \beta = 14^\circ$. The zero-quantum coherences are shifted during τ_m period using $\tau_m = \tau_m^o + kt_1$ (Rance *et al* 1985). From figure 2, cross-sections parallel to ω_2 are

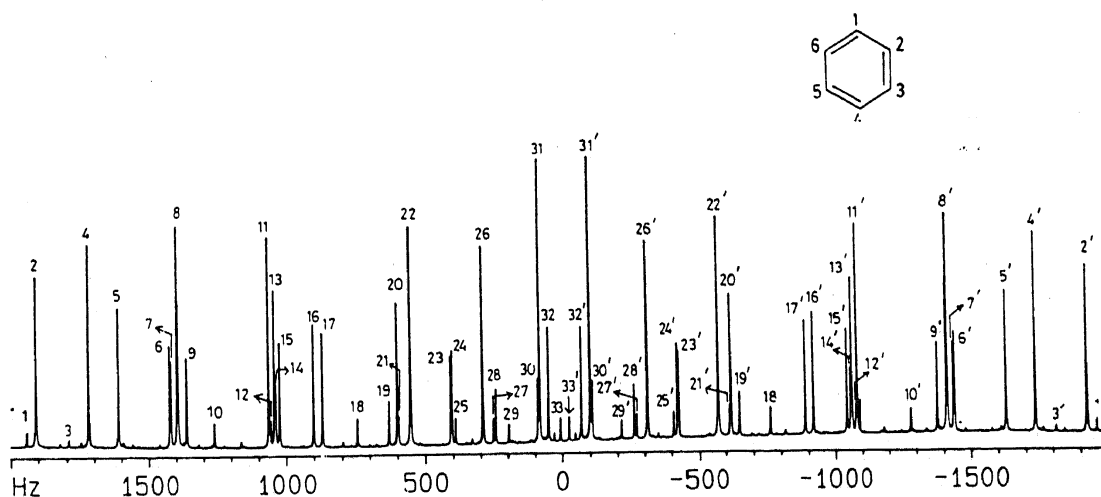


Figure 1. One-dimensional ^1H spectrum of benzene oriented in ZLI-1167. Spectrum recorded on AMX-400 MHz spectrometer.

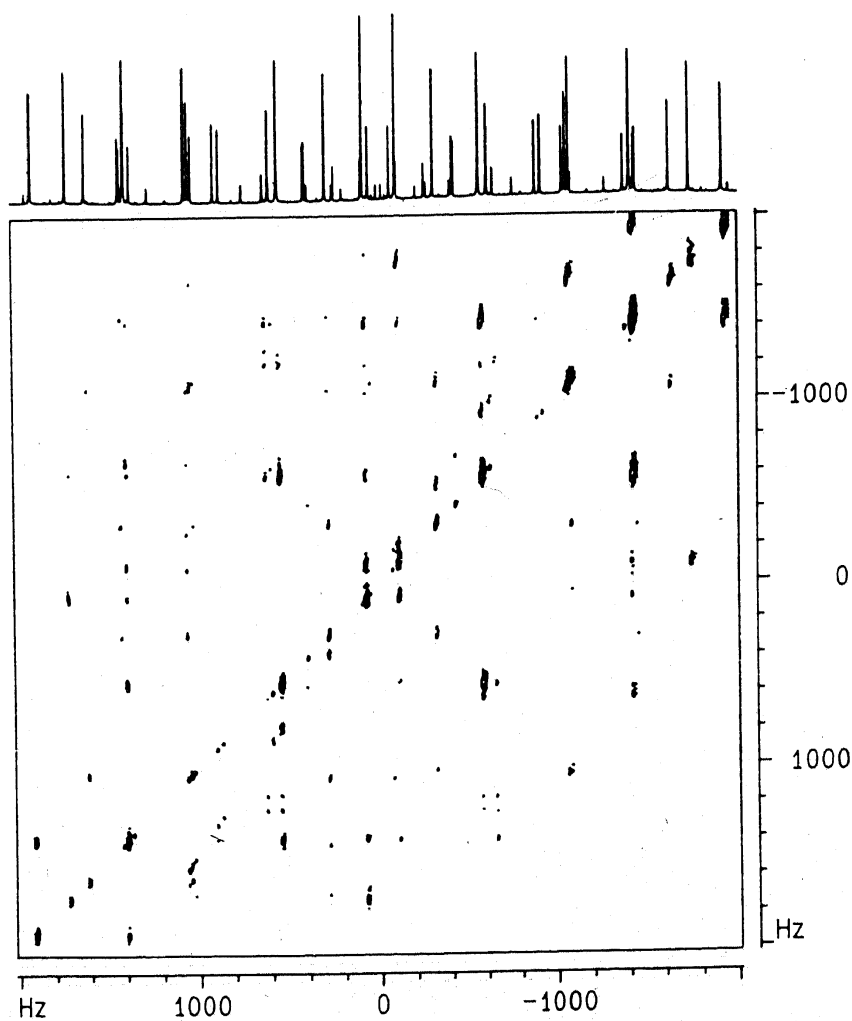


Figure 2. Two-dimensional modified Z-COSY spectrum of benzene in ZLI-1167 with $\alpha = \beta = 14^\circ$. Zero-quantum coherences are shifted along the ω_1 dimension using $\tau_m = \tau_m^0 + kt_1$; $\tau_m^0 = 20 \mu\text{s}$ and $k = 2$. $512 \times 1\text{K}$ data were acquired using AMX-400 MHz spectrometer with 8 scans per transient and zero-filled to $1\text{K} \times 1\text{K}$ before Fourier transformation.

taken at the various 1-D frequencies along ω_1 . From these cross-sections connectivity information between various transitions is obtained. The A_2 representation has a single spin state and no transition. B_1 representation has only two transitions symmetrically placed about the centre of the spectrum (Rukmani and Anil Kumar 1987). Transitions 7 and 7' are mutually connected and not connected to any other transition. Hence they belong to this representation. The difference between the frequencies of these two transitions is analytical (Saupe 1965) and is given by $|-6D_{12} + 3D_{14}|$. B_2 representation has 6 transitions (5, 13, 14, 14', 13', 5'). In this representation transitions (13, 14) and (13', 14') are too close to give resolvable cross-peaks in the 2-D spectrum (figure 1). The diagonal peaks at these frequencies are unresolved but significant in intensity, indicating that they are mutually regressively connected. There is a progressive connectivity from 13 and 14 to 13' and 14'. 5 is progressively connected to 13 and 14 and 5' to 13' and 14'. The total connectivity matrix for this representation after numbering the transitions in the order given above is,

$$C_{B_2} = \begin{bmatrix} 0 & +1 & +1 & 0 & 0 & 0 \\ +1 & 0 & -1 & 0 & +1 & 0 \\ +1 & -1 & 0 & +1 & 0 & 0 \\ 0 & 0 & +1 & 0 & -1 & +1 \\ 0 & +1 & 0 & -1 & 0 & +1 \\ 0 & 0 & 0 & +1 & +1 & 0 \end{bmatrix} \quad (1)$$

Since benzene yields a spectrum which is symmetrical about the centre, the energy level diagram also has the same symmetry. Using this information and the above connectivity matrix one obtains the energy-level diagram shown in figure 3a. In this domain the frequency difference between the transitions 5 and 5' is analytically known and is given by $|-6D_{12} - 3D_{14}|$. Another pair of transitions whose frequencies can be expressed analytically belongs to the A_1 representation. This representation has 22 transitions out of which only 2, 8, 22, 22', 8', 2' have large intensities. They showed the following connectivity among one another and the transitions are numbered in the above order.

$$C_{A_1} = \begin{bmatrix} 0 & +1 & 0 & 0 & 0 & 0 \\ +1 & 0 & +1 & 0 & 0 & 0 \\ 0 & +1 & 0 & +1 & 0 & 0 \\ 0 & 0 & +1 & 0 & +1 & 0 \\ 0 & 0 & 0 & +1 & 0 & +1 \\ 0 & 0 & 0 & 0 & +1 & 0 \end{bmatrix} \quad (2)$$

From this matrix a part of the energy-level diagram is obtained using ELEV and is shown in figure 3b. Two unique transitions from the extreme energy levels in this domain are identified as 2 and 2'. Their frequencies are analytical and the difference in frequency between these two transitions is given by $|-6D_{12} - 6D_{13} - 3D_{14}|$. Since the three transitions for which exact analytical expressions available are identified, the three dipolar couplings can be obtained without any assumptions and are $D_{12} = 472.1$ Hz, $D_{13} = 91.6$ Hz and $D_{14} = 59.7$ Hz. Within experimental error these

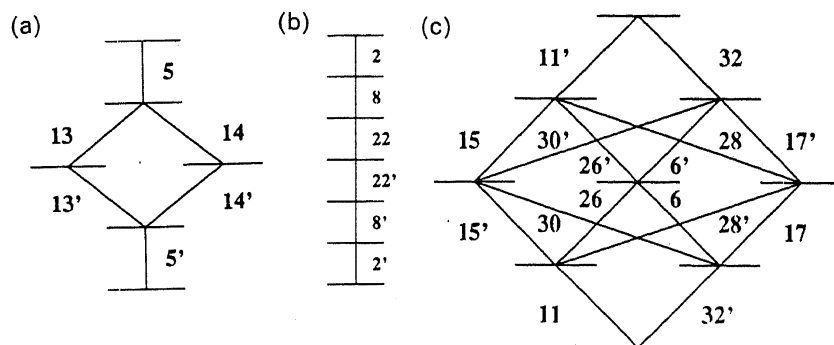


Figure 3. Energy level diagrams using the connectivity matrices and ELEV program for (a) B_2 , (b) A_1 (only a part is shown), and (c) E_1 representations. The numbering of the transitions is the same as in figure 1.

dipolar couplings indicate that oriented benzene retains regular hexagonal geometry.

The information on the J coupling is obtained in two steps. In the first step, the approximate frequencies of levels 8, 22, 22' and 8' belonging to A_1 representation can be obtained by using the approximation (a), neglecting the J 's from the off-diagonal elements of the Hamiltonian matrix, using a second-order perturbation calculation and assuming that the dipolar couplings are large compared to the J 's. This yields the difference frequency between transitions 8 and 8' as, $|-0.42J_{12} - 0.7J_{13} - 0.36J_{14} - 5.51D_{12}|$ and the difference frequency between transitions 22 and 22' as, $|-0.82J_{12} + 0.3J_{13} - 0.72J_{14} - 2.2D_{12}|$ (Saupe 1965). The experimental values obtained for these are -2609.64 Hz and -1045.22 Hz. To obtain the J values one needs the frequency of one more pair of transitions. For this the connectivity information of the E_1 representation has been utilized.

The E_1 domain has 16 transitions namely (6, 11, 15, 17, 26, 28, 30, 32, 32', 30', 28', 26', 17', 15', 11', 6'). All these transitions have significant intensity in the 1-D spectrum and it is easy to pickup these transitions in the modified Z-COSY spectrum. Numbering the transitions in the order given above, the connectivity matrix for this representation is obtained as equation (3). The energy-level diagram which ELEV gives using this matrix is shown in figure 3c. Using this diagram and the approximations mentioned above, the frequency differences between transitions 11 and 11' and 32 and 32' are given by $|-1.3J_{12} - 4.19D_{12}|$ and $|-2.7J_{12} + 0.29D_{12}|$. The numerical values obtained from the experiment for these differences are given by -1992.81 Hz and 110.3 Hz. Using this and earlier derived information the various J 's are obtained as, $J_{12} = 9.68$ Hz, $J_{13} = 3.82$ Hz and $J_{14} = -1.72$ Hz. These J values have been obtained by neglecting their contributions to the off-diagonal elements of the Hamiltonian matrix and by assuming a regular hexagonal geometry for oriented benzene. In the second step, these parameters are refined using the iterative computer program PANIC in which the above determined dipolar couplings and J values are used as starting parameters. The convergent values obtained from this program are: $D_{12} = 472.15 \pm 0.01$ Hz, $D_{13} = 91.63 \pm 0.01$ Hz, $D_{14} = 59.72 \pm 0.01$ Hz, $J_{12} = 7.52 \pm 0.01$ Hz, $J_{13} = 1.34 \pm 0.02$ Hz and $J_{14} = 0.66 \pm 0.02$ Hz. The dipolar couplings given above are the same as the input values within experimental error.

$$C_{E_1} = \begin{bmatrix} 0 & 0 & 0 & -1 & -1 & 0 & -1 & 0 & +1 & 0 & 0 & +1 & 0 & 0 & 0 & +1 \\ 0 & 0 & 0 & 0 & +1 & 0 & 0 & 0 & -1 & 0 & +1 & 0 & 0 & +1 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & -1 & +1 & 0 & 0 & -1 & 0 & -1 & 0 & +1 & +1 & 0 \\ -1 & 0 & 0 & 0 & 0 & +1 & -1 & 0 & +1 & 0 & -1 & 0 & +1 & 0 & 0 & 0 \\ -1 & +1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & -1 & +1 & 0 & -1 & 0 & +1 \\ 0 & 0 & -1 & +1 & 0 & 0 & 0 & 0 & 0 & 0 & +1 & -1 & -1 & 0 & +1 & 0 \\ -1 & 0 & +1 & -1 & 0 & 0 & 0 & 0 & +1 & +1 & 0 & 0 & 0 & -1 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & +1 & 0 & 0 & +1 & 0 & -1 & +1 \\ +1 & -1 & 0 & +1 & 0 & 0 & +1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & -1 & 0 & 0 & 0 & +1 & +1 & 0 & 0 & 0 & 0 & -1 & +1 & 0 & -1 \\ 0 & +1 & 0 & -1 & -1 & +1 & 0 & 0 & 0 & 0 & 0 & 0 & +1 & -1 & 0 & 0 \\ +1 & 0 & -1 & 0 & +1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & +1 & -1 \\ 0 & 0 & 0 & +1 & 0 & -1 & 0 & +1 & 0 & -1 & +1 & 0 & 0 & 0 & 0 & -1 \\ 0 & +1 & +1 & 0 & -1 & 0 & -1 & 0 & 0 & +1 & -1 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & +1 & 0 & 0 & +1 & 0 & -1 & 0 & 0 & 0 & +1 & 0 & 0 & 0 & 0 \\ +1 & 0 & 0 & 0 & +1 & 0 & 0 & +1 & 0 & -1 & 0 & -1 & -1 & 0 & 0 & 0 \end{bmatrix} \quad (3)$$

3. Conclusions

The two-dimensional modified Z-COSY experiment carried out on oriented benzene yields information about the connectivity of various transitions. Using this information the energy level diagrams of different irreducible representations of this molecule are constructed. On identifying the transitions, the parameters of the spin Hamiltonian are obtained. The ratios of the dipolar couplings obtained are consistent with a regular hexagon geometry, in conformity with earlier results (Saupe 1965).

References

- Anderson W A, Freeman R and Reilly C A 1963 *J. Chem. Phys.* **39** 1518
 Anil Kumar and Gordon S L 1971 *J. Chem. Phys.* **54** 3207
 Aue W P, Bartholdi E and Ernst R R 1976 *J. Chem. Phys.* **64** 2229
 Bax A 1982 *Two-dimensional nuclear magnetic resonance in liquids* (Dordrecht: Delft University Press)
 Bax A and Freeman R 1981 *J. Magn. Reson.* **44** 542
 Bodenhausen G, Vold R L and Vold R R 1980 *J. Magn. Reson.* **37** 93
 Braunschweiler L, Bodenhausen G and Ernst R R 1983 *Mol. Phys.* **48** 535
 Chandrasekher K and Anil Kumar 1988 *Magn. Reson. Chem.* **26** 821
 Drobny G 1984 *Chem. Phys. Lett* **109** 132
 Drobny G 1985 *Annu. Rev. Phys. Chem.* **36** 451
 Drobny G, Pines A, Sinton S, Weitekamp D and Wemmer D 1979 *Faraday Div. Chem. Soc. Symp.* **13** 49
 Ernst R R, Bodenhausen G and Wokaun A 1991 *Principles of nuclear magnetic resonance in one and two dimensions* (Oxford: Oxford Science Publication)
 Freeman R, Wittekoek S and Ernst R R 1970 *J. Chem. Phys.* **52** 1529
 Grace C R R and Anil Kumar 1992 *J. Magn. Reson.* **99** 81
 Grace C R R, Suryaprakash N, Anil Kumar and Khetrpal C L 1994 *J. Magn. Reson. A* **107** 79
 Griesinger C, Sørensen O W and Ernst R R 1985 *J. Am. Chem. Soc.* **107** 6394

- Griesinger C, Sørensen O W and Ernst R R 1986 *J. Chem. Phys.* **85** 6837
- Hoffman R A and Forsen S 1966 *Prog. Nucl. Magn. Reson. Spectrosc.* **1** 15
- Jakobsen H J, Linde S A and Sørensen S 1974 *J. Magn. Reson.* **15** 385
- Khetrpal C L and Diehl P 1969 *NMR basic principles and progress* (eds) P Diehl, E Fluck and A Kosfeld (Heidelberg: Springer-Verlag) Vol. 1 pp. 1-95
- Meier B U, Bodenhausen G and Ernst R R 1984 *J. Magn. Reson.* **60** 161
- Mueller L 1987 *J. Magn. Reson.* **72** 191
- Oschkinat H, Pastore A, Pfändler P and Bodenhausen G 1986 *J. Magn. Reson.* **69** 559
- Pachler K G R and Wessels P L 1973 *J. Magn. Reson.* **12** 337
- Pfändler P and Bodenhausen G 1991 *J. Magn. Reson.* **91** 65
- Piantini U, Sørensen O W and Ernst R R 1982 *J. Am. Chem. Soc.* **104** 6800
- Rance M, Bodenhausen G, Wagner G, Wüthrich K and Ernst R R 1985 *J. Magn. Reson.* **62** 497
- Rance M, Sørensen O W, Bodenhausen G, Wagner G, Ernst R R and Wüthrich K 1983 *Biochem. Biophys. Res. Commun.* **117** 479
- Rukmani K and Anil Kumar 1987 *Chem. Phys. Lett.* **133** 485
- Saube A 1965 *Z. Naturforsch.* **20a** 572
- Sinton S and Pines A 1980 *Chem. Phys. Lett.* **76** 263
- Sørensen S, Hansen R S and Jakobsen H J 1974 *J. Magn. Reson.* **14** 243
- Warren W S, Weitekamp D P and Pines A 1980 *J. Chem. Phys.* **73** 2084
- Weitekamp D P 1983 *Adv. Magn. Reson.* **11** 111