DIRECT OBSERVATION OF FIVE BOND ^1H–^1H COUPLING CORRELATION BY 2-D NMR SPECTROSCOPY AS AN AID IN RESONANCE ASSIGNMENT

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ABSTRACT

Five bond proton-proton coupling correlation between the protons of a methoxyl group and a proton on the contiguous carbon atom (H-C=C-O-C$\overset{H}{\underset{H}{<}}$H) has been observed for the first time using 2-dimensional supercosy technique[1]. The observation of this coupling correlation has helped in the unambiguous assignment of aromatic proton resonances in Trans Asarone, a tetra-substituted benzene derivative.

INTRODUCTION

TWO-DIMENSIONAL homonuclear shift correlated spectroscopy (COSY) has emerged as a powerful method for resonance assignment particularly for biological molecules[2–10]. In this method the coupling correlation is manifested as cross peaks between the nuclei which are scalar-coupled to each other with $J \geqslant 4$ Hz. Due to the antiphase character of the components of the cross peaks[11], the intensity of the cross peaks depends on the magnitude of the coupling constants as well as on the digital resolution. As a consequence, the cross peaks due to protons which have small magnitude of coupling constants are either of low intensity or not observed. To enhance the intensity of the cross peaks relative to that of diagonal peaks, a new pulse scheme named Supercosy has been introduced[1]. The pulse sequence used in Supercosy is 90-t_1-Δ-180-Δ-90-Δ-180-Δ-t_2, where Δ is a fixed delay and t_1 and t_2 are the evolution and detection periods. The theoretical background and advantages of Supercosy were discussed by Kumar *et al*[1]. By changing the value of Δ, J-tuning of Supercosy has been demonstrated to be a useful tool in delineating the long-range couplings[12,13]. Since the value of Δ in the Supercosy pulse sequence depends on the magnitude of J, one can tune the experiment to a particular value of J which may be of special interest.

In this paper, we demonstrate the use of COSY in conjunction with Supercosy for the unambiguous assignment of resonances from the para protons of Trans Asarone (1,2,4-trimethoxy-5-propenylbenzene, see figure 1). Such protons are known to be scalar-coupled[14] with $J \leqslant 0.5$ and there is no existing aid that can help in their assignment.

EXPERIMENTAL

Trans Asarone was purchased from Sigma, USA. 1-D proton spectrum in chloroform-d solution was recorded on a Bruker 500 MHz FT-NMR spectrometer. The chemical shifts were measured relative to TMS. Since the spectrum is of first order, the NMR parameters were obtained by measuring the resonance positions.

2-D COSY experiment was performed with a data matrix of 2048×512. Sixty-four transients were collected for each experiment. The time domain data was multiplied with sine square bell and sine bell functions along t_2 and t_1 dimensions respectively before Fourier transformation. Supercosy experiment was also done under similar data size and the fixed delay Δ of 40 msec was used.

RESULTS AND DISCUSSION

1-D proton spectrum of Trans Asarone is shown in figure 1. Most of the resonances have been assigned by the combined use of 1-D spectrum and the normal COSY experiment (figure 2) and these assignments are shown in figure 1. The highest field pair of doublets at 1.89 ppm is due to methyl protons. The splitting of the methyl group resonance is a result of coupling with B and A protons (figure 1), the coupling constant being $J_{BC} = 7.0$ Hz and $J_{AC} = 1.9$ Hz. The singlets at 3.84, 3.88 and 3.95 ppm are due to three methoxy groups on the benzene ring. However, it is not possible to correlate their resonance positions with their positions on the benzene ring. The pair of overlapping quartets at 6.15 ppm are assigned to B proton which is coupled to A and C protons and the two coupling constants being $J_{AB} = 16.2$ Hz and $J_{BC} = 7.0$ Hz. The second pair of quartets at 6.65 ppm is due to A proton which is coupled to B and C protons ($J_{AB} = 16.2$ Hz, $J_{AC} = 1.9$ Hz). The distinction between B and A protons is reflected in the larger magnitude of the J_{BC} coupling and this assignment is in agreement with that of a similar molecule i.e. trans-crotonaldehyde[15]. The two singlets at 6.50 and 6.96 ppm are due to the para protons of the benzene

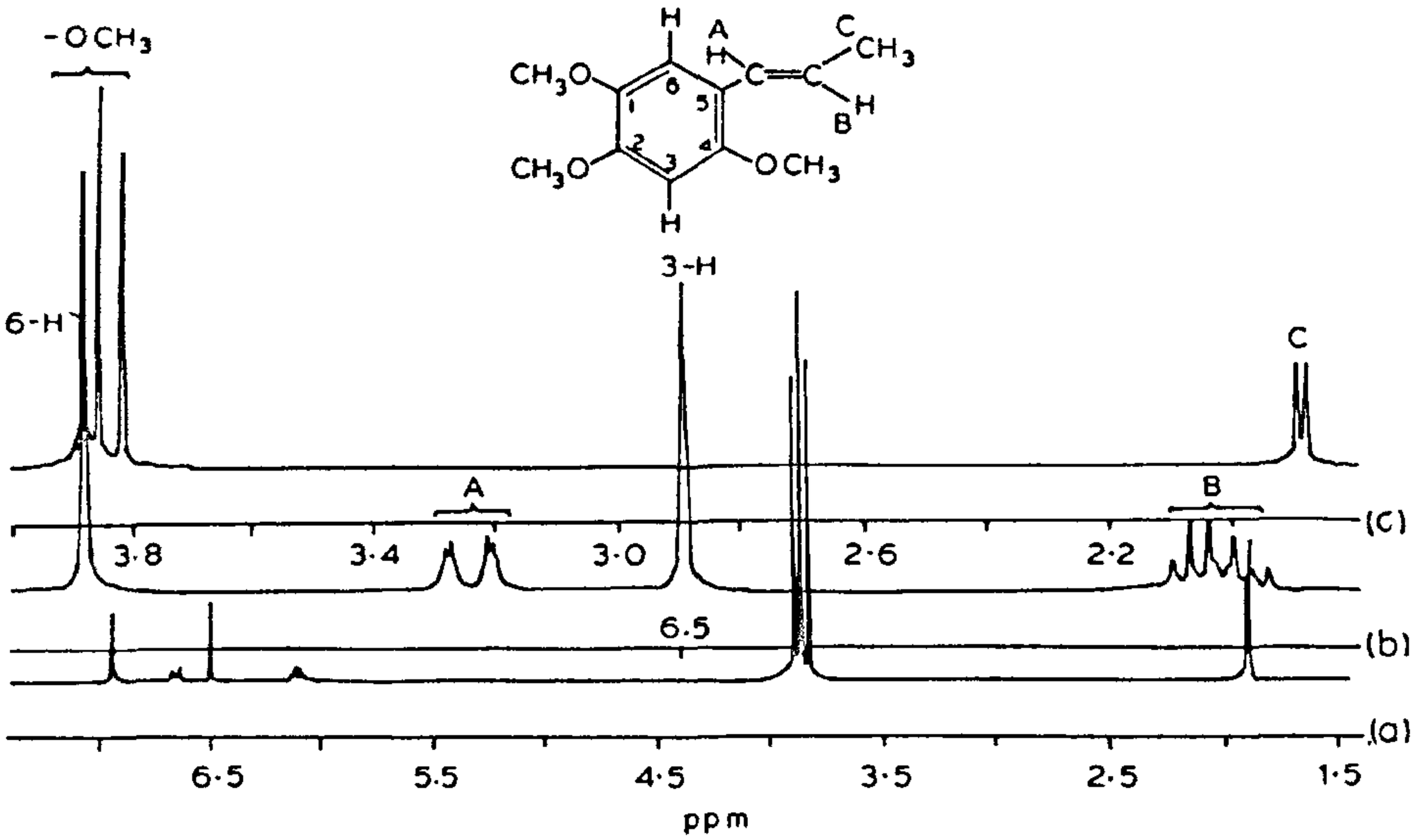

Figure 1. 500 MHz ^{1}H spectrum of Trans Asarone in chloroform-d at 300 K, conc 10 mM. **a.** Complete spectrum, **b.** blow up of region between 7 and 6 ppm, **c.** blow up of region between 4 and 1.5 ppm.

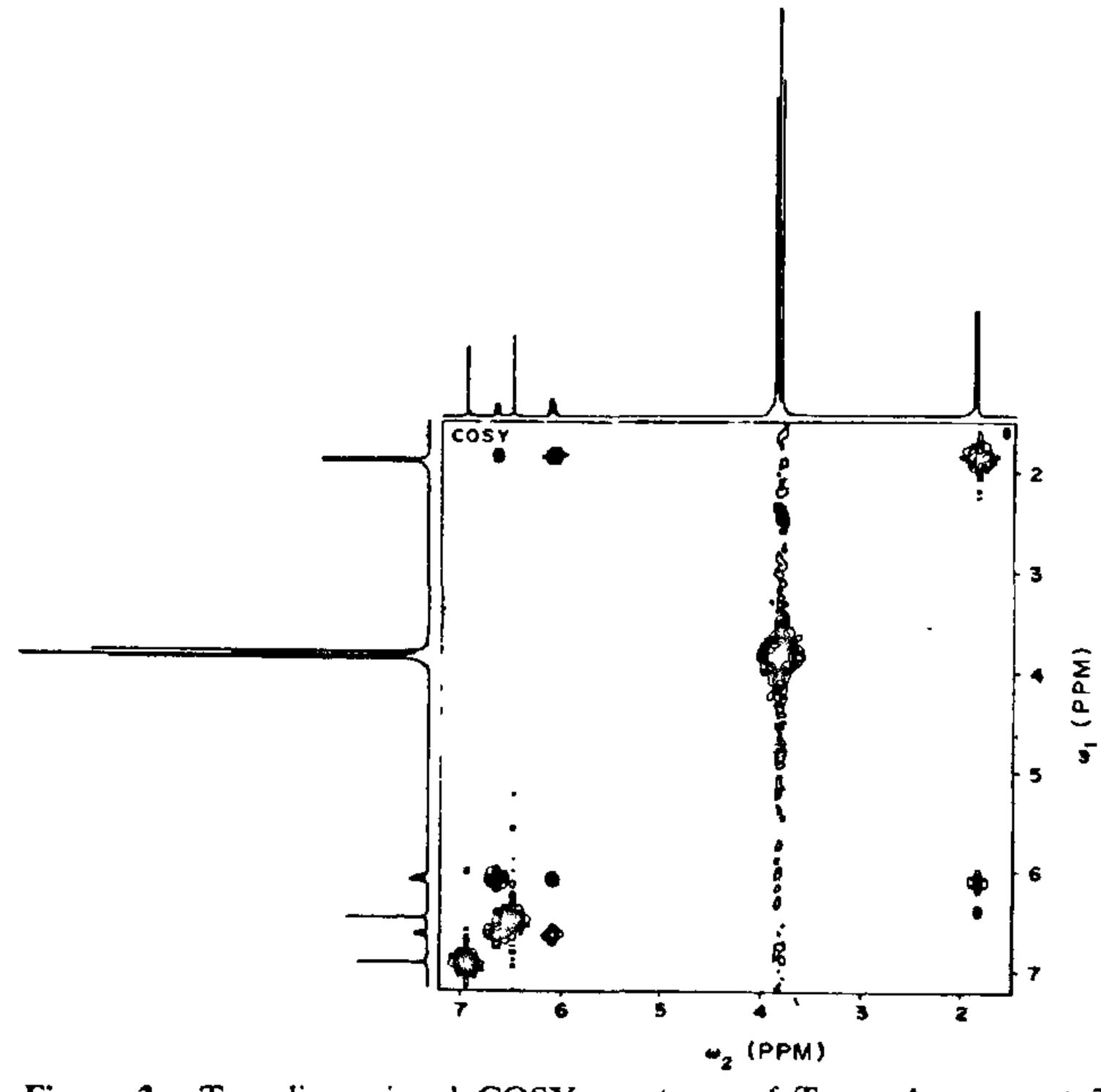

Figure 2. Two-dimensional COSY spectrum of Trans Asarone at 300 K.

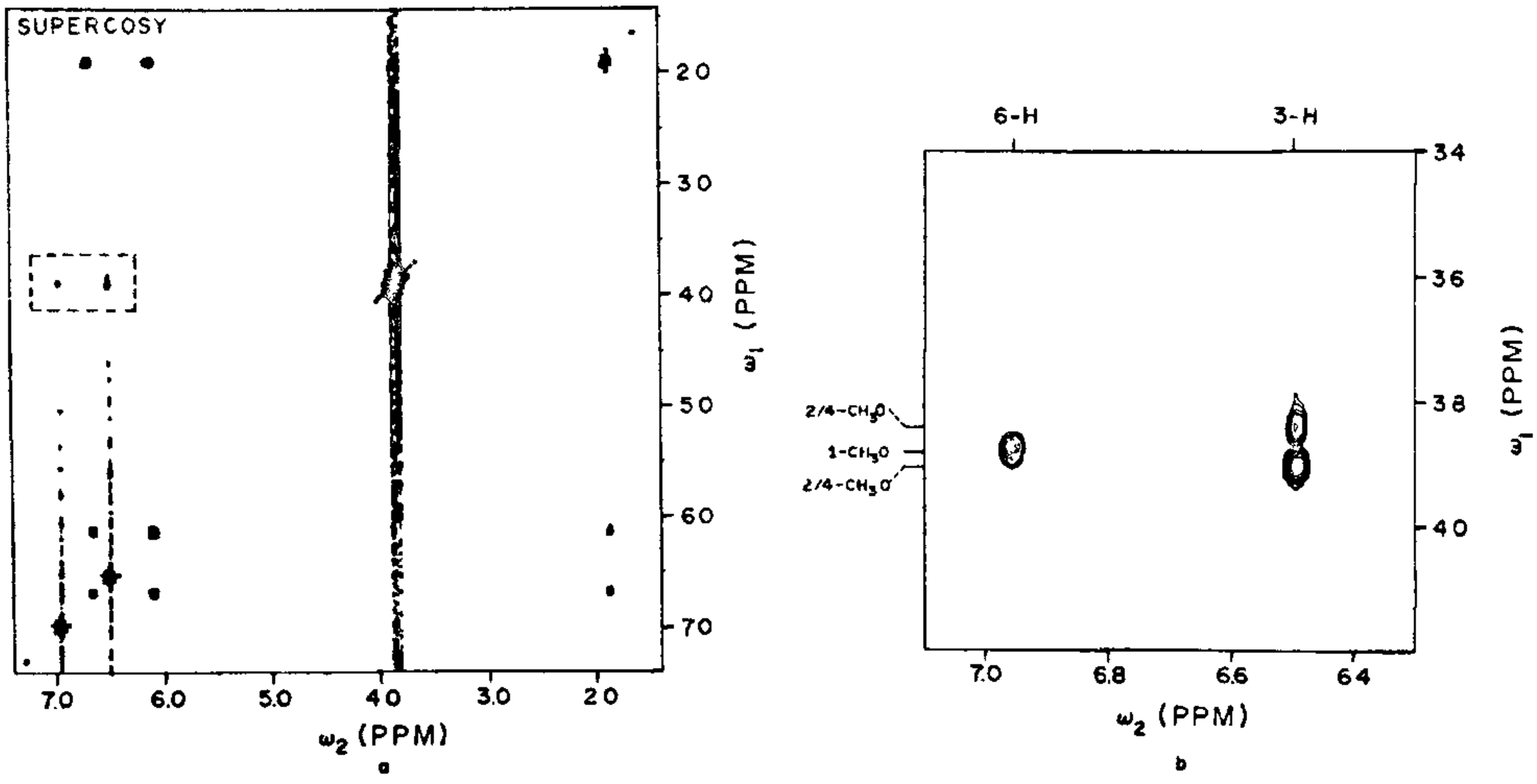

Figure 3. Two-dimensional SUPERCOSY spectrum of Trans Asarone at 300 K. Fixed Δ of 40 msec used. **a.** complete spectrum, **b.** blow up of dotted area in **a.**

ring. However, it is not possible to assign them unambiguously either from 1-D or 2-D COSY spectra i.e. whether the downfield resonance is due to proton in position 3 or 6 of the benzene ring.

The supercosy spectrum of Asarone is shown in figure 3. A comparison of the COSY (figure 2) and SUPERCOSY spectra reveals subtle differences. The cross peaks shown in the enclosed area of the supercosy spectrum are absent in the COSY spectrum. These cross peaks at the intersection of chemical shifts of methoxy and aromatic protons having ω_1 and ω_2 as 3.84 and 6.50, 3.95, 6.50 and 3.88, 6.96 ppm (shown in expanded version in figure 3b) represent the long-range coupling correlation between the aromatic protons of the benzene ring and the methoxyl groups. Two cross peaks corresponding to a single value of ω_2 i.e. 6.50 ppm and two different values of ω_1 i.e. 3.84 and 3.95 ppm correspond to methoxyl groups on carbon atoms in positions 2 and 4. These methoxyl groups are equidistant i.e. five bonds from the proton in position 3. The resonance at 6.96 ppm is correlated with methoxyl group at 3.88 ppm. Hence the peak at 3.88 ppm is assigned to methoxyl group at position 1 of the benzene ring. However, the assignment of resonances at 3.84 and 3.95 ppm to individual methoxyl groups at position 2 and 4 is not possible from these experiment.

In conclusion, the supercosy experiment has demonstrated the existence of five bond coupling between the protons of the benzene ring and the methoxyl groups on the adjacent carbon atoms and consequently their assignment.

ACKNOWLEDGEMENT

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1. Kumar, A., Hosur, R. V. and Chandrasekhar, K., *J. Magn. Reson.*, 1984, **60**, 143.
2. Kumar, A., Wagner, G., Ernst, R. R. and Wuthrich, K., *Biochem. Biophys. Res. Commun.*, 1980, **96**, 1156.
3. Wagner, G., Kumar, A. and Wuthrich, K., *Eur. J. Biochem.*, 1981, **114**, 375.
4. Wider, G., Lee, K. H. and Wuthrich, K., *J. Mol. Biol.*, 1982, **155**, 367.
5. Feigion, J., Wright, J. M., Leupin, W., Denny, W. A. and Kearns, D. R., *J. Am. Chem. Soc.*, 1982, **104**, 5540.
6. Hosur, R. V., Wider, G. and Wuthrich, K., *Eur. J. Biochem.*, 1983, **130**, 497.

7. Pardi, A., Walker, R., Rapport, H., Wider, G. and Wuthrich, K., *J. Am. Chem. Soc.*, 1983, **105**, 1652.
8. Strop, P., Wider, G. and Wuthrich, K., *J. Mol. Biol.*, 1983, **166**, 641.
9. Lown, W. J., Hanstock, C. C., Bleackley, R. C., Imbach, J. L., Rayner, B. and Vasseur, J. J., *Nucl. Acid. Res.*, 1984, **12**, 2519.
10. Frech, D., Cheng, D. M., Kau, L. S. and Tso, P. O. P., *Biochemistry*, 1983, **22**, 3194.
11. Hosur, R. V., *Curr. Sci.*, 1986, **55**, 597.
12. Mayor, S. and Hosur, R. V., *Mag. Res. Chem.*, 1985, **23**, 470.
13. Gundhi, P., Chary, K. V. R. and Hosur, R. V., *FEBS Lett.*, 1985, **191**, 92.
14. Kanekar, C. R., Govil, G. and Khetrapal, C. L., *Proc. Indian Acad. Sci.*, (*Chem. Sci.*), 1967, **A65**, 353.
15. Govil, G. and Whiffen, D. H., *Mol. Phys.*, 1967, **12**, 449.

ANNOUNCEMENT

REGIONAL (SOUTH ASIAN) SEMINAR CUM WORKSHOP ON 'DATA STORAGE, RETRIEVAL AND DISSEMINATION IN SCIENCE, WITH SPECIAL REFERENCE TO CHEMICAL AND MOLECULAR BIOSCIENCES

The National Information Centre for Crystallography (NICRYS) at the Department of Crystallography and Biophysics, University of Madras, India will organize a Seminar-cum-Workshop from 18 to 23 January 1988 on the topic above.

The purpose of the seminar is to focus attention on the large scope of modern informatics involving data storage, retrieval and dissemination and the role they play in improving the quality and grade of scientific research. Special emphasis will be given to chemical and molecular biosciences and also use of hard data bases. Besides the use of these on large computers, data base management on PC's will also receive special attention in view of its possible large scale adoption in developing countries. So also the use of colour molecular graphics will receive special attention.

The selection will be from Chemical Sciences and Molecular Biosciences. In particular Cambridge Crystallographic structural data base and a few other Crystallographic data bases will form major components. In addition some of the data bases on spectroscopy, protein structure, nucleic acid sequence etc will also be covered.

Partial list of experts involved in database creation who will cover the topics are: 1. Dr D. G. Watson, UK (Cambridge Structural data); 2. Dr G. Bergerhoff, Bonn, West Germany (Inorganic data); 3. Dr John R. Rodgers, NRC, Canada (NBS Crystal data); 4. Dr D. K. Smith/Dr R. Jenkins, JCPDS, U.S.A. (Powder diffraction data); 5. Dr G. W. A. Milne, NIH, Bethesda, U.S.A. (Chemical information system/NMR/Mass spectroscopic data); 6. Dr R. J. Feldmann, NIH, Bethesda, U.S.A. (Protein structure and molecular graphics); 7. Dr R. Blattner, University of Wisconsin, U.S.A. (Nucleic acid sequence data); 8. Dr T. F. Koetzle, B.N.L., U.S.A. (Protein data); 9. Dr B. F. Lang, CNRS, France (Gene sequence), and 10. Dr H. Bilofsky, U.S.A. (Biotechnology, data bank). Some more additions to this list are expected.

About 45 participants from India and 15 from South Asian countries will be admitted. Persons with Master's degree in Science (Physics, Biophysics, Chemistry, Biochemistry, Molecular Biology) with background knowledge of modern computers and application will be selected. Application on plain paper giving full details and biodata, including a justification for attending the workshop along with two recommendations should reach NICRYS office by 30 September 1987.

Those selected will be informed by October 1987. Address all correspondences to Prof R. Srinivasan, Honorary Director, NICRYS, Department of Crystallography and Biophysics, University of Madras, Madras 600 025.