**Figure 1.**

**Table 1** *Second order rate constants for the reaction between substituted anisoles and HBr at 80°C in 100% HOAc*

| R– | $10^2 k_2$ lit mol$^{-1}$ min$^{-1}$ |
|---|---|
| H– | 0.912 |
| 2–CH$_3$ | 2.43 |
| 3–CH$_3$ | 1.05 |
| 4–CH$_3$ | 1.25 |
| 4–COOH | 1.45 |
| 4–Cl | 1.21 |
| 4–NO$_2$ | 2.03 |
| 4–COCH$_3$ | 1.51 |

[HBr] = 9.49 × 10$^{-2}$M, [Anisole] = 5.51 × 10$^{-2}$M

variation of the mechanism would explain the right side of the plot (where electron-withdrawing substituents like a − NO$_2$ group also accelerate the reaction because the $p$ − NO$_2$–phenoxide ion being a good leaving group gets displaced in a S$_N$2 process quite easily).

20 February 1987

1. Hughes, E. D. and Ingold, C. K., *J. Chem. Soc.*, 1935, 251.

2. Wilcox Jr., C. F. and Sager, M. A., *J. Org. Chem.*, 1969, **34**, 2319.

3. Brown, R. F. and Claflin, E. F., *J. Am. Chem. Soc.*, 1958, **80**, 5960.

# HIGH-RESOLUTION SOLID-STATE CARBON-13 NUCLEAR MAGNETIC RESONANCE STUDY OF ACETAMINOPHEN: A COMMON ANALGESIC DRUG*

## N. R. JAGANNATHAN

*Solid State and Structural Chemistry Unit, Indian Institute of Science, Bangalore 560 012, India.*

A variety of analytical techniques such as, infra-red (IR) spectroscopy, differential scanning calorimetry (DSC), X-ray powder diffraction, and other such methods are available for the study of solid state chemistry of drugs. However, only a few of them allow direct analysis in the tablet form. For example, among the IR and DSC methods which have been used to characterize crystalline forms, some forms known to be different show identical IR spectra and DST properties. While, X-ray diffrac-

tion is sensitive to differences in crystal packing it is an averaging technique, not extremely useful for solid solutions or for amorphous forms. On the other hand, the high-resolution solid-state carbon-13 NMR spectroscopy appears to offer significant advantages over other techniques for the study of solid drugs, but application of this powerful method in this area is very limited[1,2].

Proton enhanced (PE) carbon-13 NMR[3], combined with high-power decoupling, and magic angle spinning (MAS)[4] gives an enormous improvement in the sensitivity and resolution of the solid state spectrum comparable to that obtained in solution. Consequently, ^{13}C NMR studies of molecules using this technique, promises to give detailed information about the electronic structure and molecular dynamics of molecules in solids. In this paper, a report on the solid-state ^{13}C NMR study of acetaminophen is presented. Acetaminophen is a white, odourless crystalline powder, possessing a bitter taste and is widely used as analgesic and antipyretic. It has different generic names such as paracetamol, acetophenum, etc.

The ^{13}C CP/MAS NMR spectra were obtained at 75.47 MHz using a Bruker MSL300 spectrometer. Spin-locked cross-polarization was established through a matched Hartmann-Hahn condition[5]. Contact time was carefully determined to obtain a maximum signal-to-noise ratio. A single contact of 1 msec was adopted throughout for all the samples. The acquisition time was usually 28 msec and a repetition time of 5 sec was used between successive pulsing. A phase alternation was used throughout to eliminate base-line and intensity artifacts. Samples were packed in an aluminium oxide cylindrical type of rotors and were spun at 3.4 kHz. Non-quaternary suppression (n.q.s.) experiment, which provides a means for discriminating between resonances of carbon atoms which bear protons from those which do not, was carried out following the procedure of Opella et al[6]. In doing this experiment, a delay time of 50 $\mu$sec was applied immediately after the proton 90° pulse and preceding the decoupling pulse. All the chemical shifts were externally referenced with respect to TMS. The solution ^{13}C NMR spectrum of acetaminophen was obtained at 67.89 MHz using a Bruker WH270 spectrometer in DMSO-d6 solvent at an ambient temperature. Two commercially available acetaminophen tablets, Crocin (Dulphar-Interfran Ltd., Vapi, Gujarat, India) and Tylenol (McNeil Consumer Products Ltd., Guleph, Ontario, Canada) were used in this investigation.
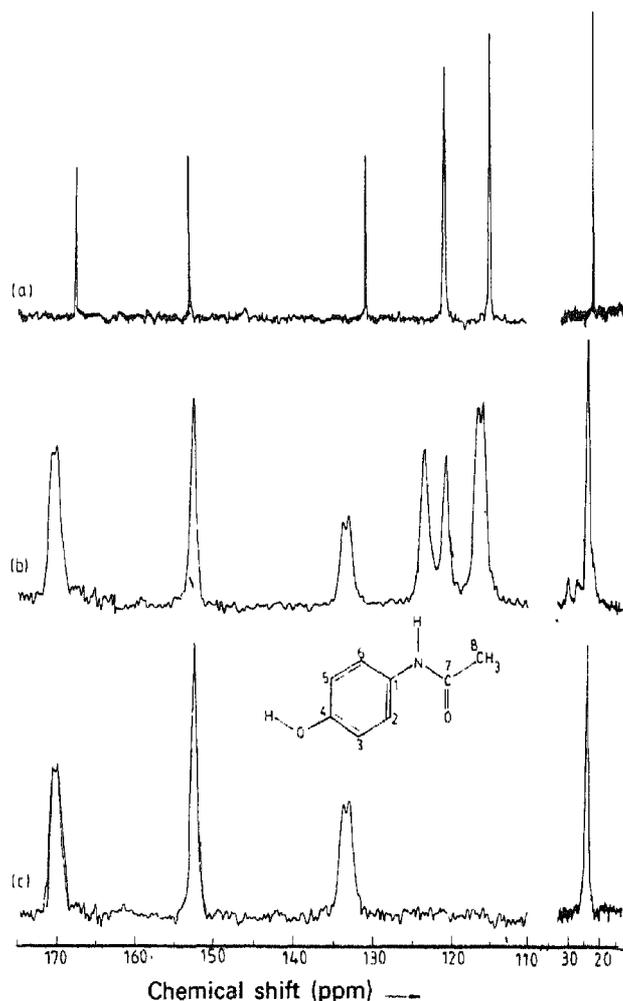
The ^{13}C NMR spectrum of acetaminophen in $d_6$-



**Figure 1.** Carbon-13 NMR spectra of acetaminophen. (a) In DMSO-$d_6$ solution at 67.89 MHz (5000 scans) recorded on a Bruker WH270 spectrometer, (b) conventional solid-state CP/MAS spectrum at 75.47 MHz recorded on a Bruker MSL300 spectrometer with 1 msec contact time and 300 scans, and (c) using non-quaternary suppression pulse scheme[6]; number of scans 300 with 1 msec contact time.

dimethylsulphoxide solvent is shown in figure 1. For assigning the different carbon lines, a proton-noise-decoupled and single frequency off-resonance decoupled spectra were taken. The multiplicities generated in off-resonance decoupled spectra enabled distinction between the different types of carbons. The chemical shift values are as given in table 1. The downfield resonance at 167.7 ppm is assigned to the carbonyl carbon C7, while the C4 carbon is assigned to the line occurring at 130.9 ppm. Carbon C1 attached to the nitrogen is assigr    to the peak at 153.1 ppm. The carbon resonances C7 and C1 could also be assigned on the basis of the solid-state ^{13}C NMR spectrum (figure 1c) which shows an asymmetric splitting of these carbon resonances due to nitrogen (see later). The electronic effect of the oxygen atom attached to the phenyl

**Table 1**  *Carbon-13 chemical shifts in acetaminophen*

| Numbering of carbon atoms | Chemical shifts (ppm) | | |
| --- | --- | --- | --- |
| | | Solid | |
| | Solution | Conventional | n.q.s.[+] |
| C1 | 130.9 | 133.8* | 133.8* |
| | | 133.1* | 133.1* |
| C2 | 121.0 | 120.8 | — |
| C3 | 115.0 | 116.5[''] | — |
| C4 | 153.1 | 152.5 | 152.5 |
| C5 | 115.0 | 116.0['''] | — |
| C6 | 121.0 | 123.6 | — |
| C7 | 167.7 | 170.3* | 170.3* |
| | | 169.8* | 169.8* |
| C8 | 23.5 | 24.0 | 24.0 |

[+] non-quarternary suppression (see ref.6); * splitting due to [14]N (see text); ['''] assignment could be reversed.

ring produces an upfield shift of the ortho carbons C3 and C5 and hence they are assigned to the upfield resonance at 115 ppm. This leaves us with the assignment of C2 and C6 carbon resonances to the line at 121 ppm.

The conventional ^{13}C CP/MAS NMR spectrum and that obtained by employing the n.q.s. pulse scheme are also shown in figure 1. The immediate interpretation of the dipolar dephasing experiment is that the ^{13}C carbon resonances of carbons C1, C4, C7 and C8 contribute to the lines at 133, 152.5, 170.3 and 24.0 ppm. We notice that the resonances of carbons C1 and C7 are split into asymmetric doublets characterstic of [13]C–[14]N dipolar couplings in high-resolution solid-state spectra. This unusual line shape results from the failure of magic angle spinning to completely average out the [13]C–[14]N dipolar interaction. The split is defined as the center of mass of the more intense component (corresponding to the $n = \pm 1$ state of ^{14}N) minus the center of mass of the less intense component (corresponding to the $n = 0$ state) of the asymmetric doublet. The dipolar splits observed in this molecule are approximately $-54.9$ and $-42.7$ Hz respectively for the C1 and C7 carbons.

In solution, the ring carbon resonances C2 and C6 overlap, whereas in the solid state they are clearly well separated. X-ray structure[7] indicates that this is due to the fixed orientation of the group attached to the C-N bond with respect to the ring. Whereas free rotation of this group may occur in solution, the molecule is locked in the crystalline state such that

C2 and C6 carbons are no longer equivalent. This difference has been detected by solid-state NMR as a doublet with a 2.8 ppm splitting. It is possible from the structural details to assign the low-frequency peak of the doublet to C2 (120.8 ppm) and the high-frequency peak (123.6 ppm) to C6 carbons. Similarly, the resonances of C3 and C5 carbons are found to overlap in solution but in the solid state they occur as a sharp doublet. Again, this is due to fact that these carbons are no longer equivalent in the solid state and from the X-ray structure C3 carbon is assigned to the peak at 116.5 ppm, while C5 carbon to the peak at 116.0 ppm.

There are reports that commercially available drugs of the same kind have different absorption and excreation values[8,9]. In order to examine this aspect w.r.t. acetaminophen-based drugs, solid-state ^{13}C NMR spectra of two commercially available paracetamol tablets, namely on Crocin and Tylenol (extra- and regular strength) were recorded. No significant difference in their spectral pattern was noticed, except for the different content of the binder used in the tablet formation (figure 2). These indicate that there were no interactions between the drug and the binder components. This means that the tablets examined must be formulated by dry mixing of all the consitituents such as binders etc in the solid form.

It is known that crystallization of acetaminophen from a wide range of solvents gives different
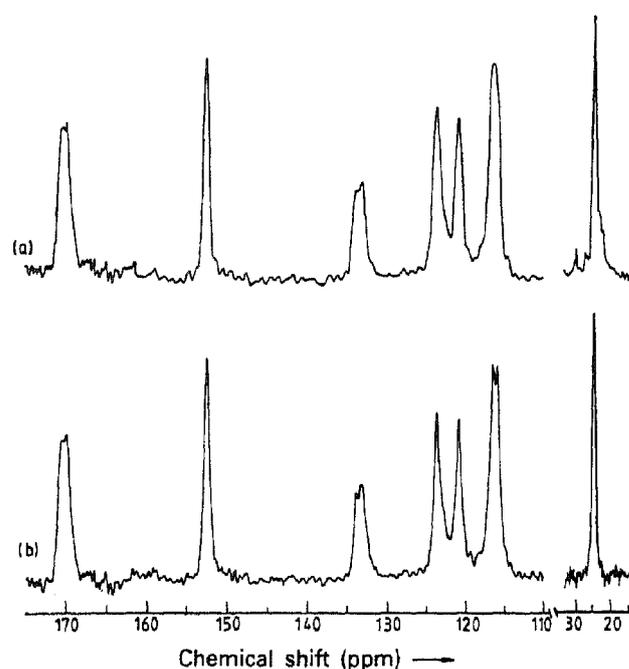


**Figure 2.** Solid-state ^{13}C NMR spectra of (a) Crocin, and (b) Tylenol (extra-strength) tablets. About 300 scans were accumulated for each, with 1 msec contact time.

polymorphic forms[10]. Preliminary solid-state ^{13}C NMR spectra run on two samples crystallized from water and acetonitrile failed to show any significant changes in the carbon signals. This result is in accordance with the DSC, IR and X-ray diffraction (powder) results[10]. Further work on these lines is in progress.

26 February 1987

1. Byrn, S. R., *Solid-state chemistry of drugs*, Academic Press, New York, 1982.

2. Chang, C., Diaz, L. E., Morin, F. and Grant, D. M., *Magn. Reson. Chem.*, 1986, **24**, 768.

3. Pines, A., Gibby, M. G. and Waugh, J. S., *J. Chem. Phys.*, 1973, **59**, 569.

4. Andrew, E. R., *Prog. Nucl. Magn. Reson. Spectrosc.*, 1971, **8**, 1.

5. Hartmann, S. R. and Hahn, E. L., *Phys. Rev.*, 1962, **128**, 2042.

6. Opella, S. J. and Frey, M. H., *J. Am. Chem. Soc.*, 1979, **101**, 5854.

7. Haisa, H., Kashino, S. and Maeda, H., *Acta Crystallogr.*, 1974, **B30**, 2510.

8. Nogami, H. and Kato, Y., *Nihon Yakuzaishi-Kyokai Ziasshi*, 1955, **7**, 152.

9. Kato, Y., Togawa, S. and Ishii, K., *Yakuzaigaku*, 1973, **33**, 185.

10. Fairbrother, J. E., In: *Analytical profiles of drug substances* (ed.) K. Florey, Academic Press, New York, 1974, Vol.3.

## XYLANASE PRODUCTION BY *FUSARIUM OXYSPORUM* F.SP. *UDUM*

G. S. PRASAD, K. PRABAKARAN* and H. C. DUBE

*Department of Life Sciences, Bhavnagar University, Bhavnagar 364 002, India.*
**Present address: A. N. Bach Institute of Biochemistry, USSR Academy of Sciences, Moscow, USSR.*

XYLOGLUCANS and xylans are the most common hemicelluloses. The former is the major component ⟩ of the primary cell wall of plants, while the t mostly in the secondary wall of These form a protective covering ibrils preventing enzymatic de-

gradation of cellulose[1,2]. The xylanases include (i) endo-xylanase (EC 3.2.1.8) which attacks the xylan chain randomly releasing xylooligosaccharides, and (ii) β-xylosidase (EC 3.2.1.37) which attacks terminally releasing xylose residues[3].

Xylanases are used in the food processing industry for isolation of proteinaceous and other subcellular materials from plants[4], pretreatment of agricultural wastes to enhance cellulose degradation[5] by removing the overlying xyloglucan molecules, and also in paper industries to remove hemicelluloses from paper pulp to produce high grade dissolving pulps. The present paper examines xylanase production by *Fusarium oxysporum* f.sp. *udum*, causing wilt of pigeon pea.

The organism was grown in 25 ml of Mandels' medium[6] supplemented with 0.5% larch-wood xylan (Sigma Chemical Co., USA) in 100 ml Erlenmeyer flasks. The pH of the medium was adjusted to 5.2. The flasks seeded with 1 ml spore suspension ($5 \times 10^6$ spores/ml) collected from culture grown on Czapek medium containing glucose were incubated at 28°C without shaking for 14 days. On alternate days the culture media were centrifuged at 10,000 rpm for 15 min at 4°C, dialyzed against several volumes of distilled water overnight at 4°C and used as the enzyme sample. All enzyme assays were carried out at 45°C and at pH 7.0, using 0.05 M potassium phosphate buffer. For endo-xylanase assay 0.5 ml of the dialyzed enzyme sample was allowed to react with 0.5 ml of substrate (0.5% larch-wood xylan) for 30 min and the release of reducing groups was measured by Miller's method[7]. The activity is expressed as $\mu$mol of xylose released/ ml/min. β-xylosidase activity was assayed by monitoring the release of paranitrophenol from PNPX (para nitriphenyl β-D-xylopyranoside) (20 mM) (Sigma Chemical Co., USA). The reaction mixture containing 1 ml of enzyme and 1 ml of substrate was allowed to react for 1 hr. The reaction was stopped by adding 2 ml of 1 M sodium carbonate and 10 ml of distilled water and the absorbance of the mixture was noted at 405 nm[8]. Results are expressed as $\mu$mol of paranitrophenol released/hr/ml of enzyme.

Table 1 shows that the organism produced both endo-xylanase and β-xylosidase from the second day onwards. The activity of endo-xylanase in the growth medium increased sharply showing optimum value on the 8th day. β-xylosidase, which was also optimally active on the 8th day, had rather a slow but steady increase as compared to endo-xylanase. This could be due to the sequential mode of action of these enzymes on xylan. Chromatographic analy-