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## NMR chemical shielding tensors as a tool for probing molecular environment: Carbon-13 NMR and X-ray studies on malonic acid

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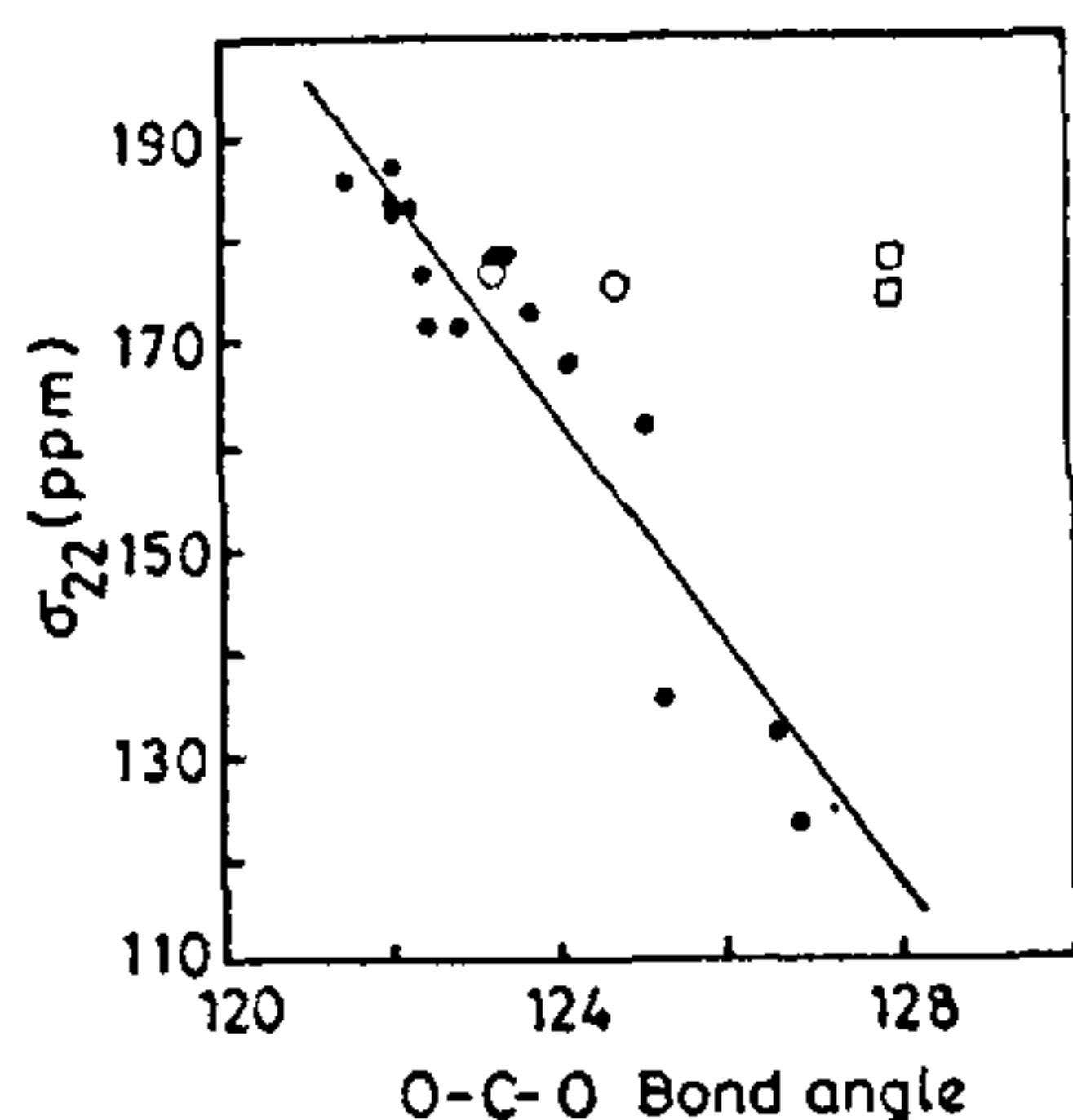
An empirical correlation proposed earlier<sup>1</sup> between the  $\sigma_{22}$  component of the chemical shielding tensor and the O-C-O bond angle at the carboxyl carbon in several dicarboxylic acids and related compounds, formed the basis for the reinvestigation of the X-ray structure of malonic acid. The X-ray analysis provides a striking confirmation of the correlation proposed and establishes the fact that the chemical shielding tensors can provide a sensitive tool for determining the local molecular geometry.

THE magnetic shielding tensors  $\sigma$  determined by proton enhanced rare spin NMR methods have provided a wealth of structural information about solid state conformation of molecules<sup>2-5</sup>. This information is more detailed than that obtained from NMR experiments involving the liquid state since it enables the determination of the principal values of the shielding tensor rather than just the trace of the tensor, thus providing more information on the chemical environment. A knowledge of the individual components of  $\sigma$ , which are likely to be more sensitive to geometrical

changes than their isotropic counterparts, should lead to a better understanding of the mechanisms responsible for the chemical shift.

It has often been reported in the literature that the  $\sigma$  component of the carboxylic carbon tensor is extremely sensitive to the local structure<sup>3</sup>. Recently, a linear correlation was proposed between the  $\sigma_{22}$  component of the carboxyl carbon tensor and the O-C-O bond angle at this carbon atom<sup>1</sup>. Figure 1 shows such a correlation for a group of organic compounds containing carboxyl carbon as one of their functional groups, with a correlation coefficient of 0.94. The points of the curve could be fitted to a linear function of the form  $y = mx + c$  with  $m = -11.3$  and  $c = 1566$ . For compounds with a known bond angle, this equation could be used to obtain an approximate estimate of the  $\sigma_{22}$  value of the tensor or vice versa. Malonic acid whose X-ray structure is known<sup>6</sup>, was excluded from the above calculations, since application of this relationship to malonic acid using the conformational parameters (i.e., O-C-O = 128° for both carboxyl groups) reported in the X-ray studies, yields a value of 120 ppm for  $\sigma_{22}$  for both the carboxyl carbons which is very much at variance with the value of 178 and 175 ppm observed (indicated by □ in Figure 1) from NMR experiments. Consequently, we decided to reinvestigate the X-ray structure of malonic acid, primarily to improve the accuracy of the X-ray analysis and also to test the potential utility of the empirical relationship established between the  $\sigma_{22}$  and the O-C-O bond angle.

Complete three-dimensional CuK $\alpha$  intensity data (to



**Figure 1.** Linear plot showing correlation between the principal element tensor  $\sigma_{22}$  and the O-C-O bond angle for the carboxyl carbons for a group of organic compounds. The compounds included in (●) (solid circle) are: anhydrous oxalic acid, oxalic acid dihydrate, ammonium hydrogen oxalate hemihydrate, benzoic acid, pyromellitic acid dihydrate, succinic acid, glutaric acid, adipic acid, pimelic acid, suberic acid, potassium hydrogensuccinate, ammonium hydrogen-fumarate, potassium hydrogenmalonate, potassium hydrogenglutarate. Square (□) represents the experimental  $\sigma_{22}$  value plotted against the O-C-O bond angles of  $128^\circ$  for malonic acid from old X-ray data<sup>6</sup>. Circle (○) represents the experimental  $\sigma_{22}$  values plotted for the O-C-O bond angles of malonic acid obtained from new X-ray data<sup>7</sup>.

a limit of  $\sin \theta/\lambda < 0.6 \text{ \AA}^{-1}$ ) were collected on an Enraf-Nonius CAD-4 automated computer-controlled diffractometer equipped with a scintillation counter. The structure was solved by direct methods and refined to a final R factor of 0.039 (ref. 7). The O-C-O bond angles for the two carboxyl groups, as determined by the new X-ray data are found to be  $123.3^\circ$  and  $124.8^\circ$ . Substitution of these into the linear equation gives  $\sigma_{22}$  values for the two carboxyl carbons as 173 and 156 ppm respectively, in close agreement with the observed  $\sigma_{22}$  values of 178 and 175 ppm with a margin of  $\pm 7$  ppm in the experimental measurements<sup>1</sup> (as indicated by a circle (○) in Figure 1).

It should be emphasized here that even though the correlation is purely empirical, the present study illustrates clearly, that the chemical shielding tensors (CST) obtained from solid state NMR studies of organic molecules are very sensitive to the local geometry and symmetry of the atoms involved. It may be noted that the principal values of the CST from NMR experiments arise mainly due to the anisotropic nature of the electron density distribution surrounding the nucleus. Since X-ray crystallographic analysis also deals with electron density distribution around atoms, these types of correlations may share a common physical basis. The study of solid state structures of organic and other compounds using the combined techniques of solid state NMR and X-ray crystallography is thus

likely to provide us a unique opportunity to correlate chemical shielding effects with the molecular environment of the nucleus of interest.

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## Nonspecific defence mechanisms of *Cirrhinus mrigala* against *Escherichia coli* and *Salmonella typhi*

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As a part of our studies on the microbiology of water and fish in East Calcutta sewage processing oxidation ponds, this paper reports on the phagocytic defense mechanism of the fish *Cirrhinus mrigala* against some sewage-borne bacteria like *Escherichia coli* and *Salmonella typhi*. These bacteria are found in large concentrations in the sewage-fed ponds. They should be potential human pathogens but are non-pathogenic for this fish. The minimum inhibitory titer and minimum bactericidal titer of the fish serum were higher in case of *S. typhi* than *E. coli*. The progress of phagocytosis of both bacteria by fish blood followed similar time course.

SINCE 1930, Calcutta municipal sewage comprising mainly human and animal wastes<sup>1</sup> has been in use for pisciculture in 7,500 acres of water bodies without being subjected to any pretreatment by mechanical and chemical means. In fact the practice of using human and animal wastes for pisciculture has been going on for a long time in Asia<sup>2</sup>. It carries a variety of human pathogens which, if incorporated in fish, may cause health hazards for consumers and handlers<sup>3-7</sup>. In recent years, a hot debate has been going on between