We immediately see the squares arising from the couplings between ^{c}P and ^{b}P and between ^{b}P and ^{a}P. But if we look closely we can see that there is also a weak correlation between ^{c}P and ^{a}P: this shows that there *is* a coupling between them, as we had expected. But because we can not see the coupling in the 1D spectrum the coupling constant must be smaller than the signal linewidth. This is one of the beauties of 2D correlation experiments: they often allow the detection of couplings which are not visible in the corresponding 1D spectra!

3 Quadrupolar Nucleus Experiments

3.1 General Principles: Quadrupole Moment, Relaxation, Linewidth

The experiments we have so far described have been used to study nuclei with spin I = ½ (^{1}H, ^{13}C, ^{31}P). Our model compounds **1** and **2** contain two further atoms (oxygen and chlorine), which have no NMR-active isotope with spin ½. Oxygen does however have an NMR-active isotope with spin I = 5/2 but very low natural abundance (0.037%): this is ^{17}O. Chlorine has two NMR-active isotopes: ^{35}Cl (I = 3/2, 75.53%) and ^{37}Cl (I = 3/2, 24.47%).

NMR-active nuclei with spin > ½ (these include, as we mentioned previously, deuterium) have an electric quadrupole moment and are thus referred to as **quadrupolar nuclei**.

These nuclei (and they form by far the majority of the NMR-active nuclei!) are subject to relaxation mechanisms which involve interactions with the quadrupole moment. The relaxation times T_1 and T_2 (T_2 is a second relaxation variable called the **spin-spin relaxation time**) of such nuclei are very short, so that very broad NMR lines are normally observed. The relaxation times, and the linewidths, depend on the symmetry of the electronic environment. If the charge distribution is spherically symmetrical the lines are sharp, but if it is ellipsoidal they are broad.

3.2 ^{17}O

Oxygen plays a central role in organic and inorganic chemistry as well as a vital role in animal and plant life. NMR studies on this element could therefore be of great interest. Although oxygen-17 has such a low natural abundance, it is possible under correctly chosen conditions to obtain high-quality NMR spectra. Thus NMR measurements on biological materials can readily be carried out. The chemical shift range is very large (around 2500 ppm), so that in spite of the large linewidths it is possible to study structural changes readily: coupling information can also often be obtained.

Briefly, the experimental conditions should be based on the following information: acetonitrile is the recommended solvent, as it gives sharper lines than

chloroform. Temperature also affects the linewidth, so that the effect of working at above room temperature should be tested. Because of the fast relaxation of the oxygen nuclei it is possible to use extremely short pulse repetition rates (50–200 msec), and the acquisition time should also be made short by appropriate choice of the number of data points and the sweep width. In this way we can record a large number of FIDs in a relatively short time. The FID needs to be subjected to exponential multiplication using linewidths of 50 to 500 Hz.

The normal reference substance is water, the signal of which is set equal to 0 ppm.

3.2.1
^{17}O Spectrum of 7: Chemical Shift (Reference), Coupling with P

We shall use our model compound **7** to show how oxygen-17 NMR can be used. Figure 31 shows the spectrum, recorded using a 40% solution of **7** in CD_3CN at a temperature of 55°C.

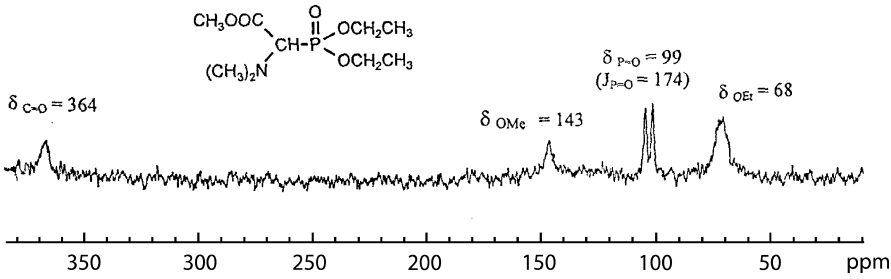

Fig. 31 Oxygen-17 spectrum for compound **7** (40% in CD_3CN, temperature 55°C)

Compound **7** contains four different oxygen nuclei, so that we expect four signals. As in carbon-13 NMR, the signal associated with the carbonyl group lies at very low field (364 ppm). The signal for the P=O oxygen at 99 ppm is immediately recognisable because of the presence of the one-bond P–O coupling (J = 174 Hz). The two remaining signals are due to the methoxy oxygen bound to carbon and the ethoxy oxygens bound to phosphorus: here the signal intensity difference indicates which is which, and the literature confirms that the high-field signal is indeed due to the ethoxy oxygens.

3.2.2
P–O Correlation

In principle it is possible (with a suitably configured spectrometer) to carry out correlation experiments between any pair of NMR-active nuclei. How-

ever, a P–O correlation is certainly not trivial, as we are dealing with a "good" (spin-½) and a "bad" (quadrupolar) nucleus. Indeed, all our attempts to carry out such an experiment failed completely.

4
HPLC-NMR Coupling

4.1
General Principles, NMR as a Highly Sensitive Analytical Tool (µg to ng Amounts)

The identification and structural characterization of biological materials, obtained for example from plants, was traditionally carried out via the classical sequence involving extraction, separation, isolation and characterization, a sequence which requires large amounts of substance and a great deal of time. Industrial problems, for example the search for small amounts of contaminants in industrial products or in waste water, also require intensive analytical studies.

A direct combination of separation and analysis techniques is thus invaluable. **GLC-MS** and **HPLC**-MS coupling are now routinely used. Because of the high sensitivity of modern NMR instruments the coupling of HPLC and NMR is now used in many NMR laboratories, and we shall discuss the principles and show some results below.

The coupling of HPLC in tandem with NMR requires two separate systems:

a) a conventional HPLC system with a standard detector (e.g. UV) and a monitoring system to observe and control the chromatography.
b) a normal NMR spectrometer with a dedicated probehead.

A long capillary with a computer-controlled switching valve (the instruments must be separated by 2–3 metres because of the strong magnetic field) connects the exit from the HPLC with the probehead. The latter is completely different in its construction from conventional probeheads: instead of the NMR tube there is a small flow cell, the volume of which is 40–100 µl. The transmitter and receiver coils are attached directly to the cell in order to maximize the sensitivity.

There are two different ways of carrying out an HPLC-NMR experiment:

a) Continuous Flow
   The NMR spectrum is recorded during the chromatographic separation. Data are collected as in a 2D experiment, the two dimensions being the chemical shift and the retention time of the chromatogram.
b) Stopped Flow
   Here the chromatographic scan is stopped at defined times and the NMR experiments then carried out. In this case it is possible to adjust the measurement time of the experiment to the concentration of the sample.

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