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Prospects for *in vivo* NMR methods in xenobiotic research in plants

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Abstract

The application of non-invasive nuclear magnetic resonance (NMR) methods in xenobiotic research is reviewed in relation to: (i) the characterisation of the effects of xenobiotics on the metabolism of plants and plant cell suspensions; (ii) the direct detection of xenobiotics and their degradation products *in vivo*; and (iii) the spatial localisation of xenobiotics and their derivatives at the subcellular and tissue levels. Novel information has been generated by *in vivo* NMR studies of both agrochemicals and heavy metals, but a lack of generality in the methods makes it difficult to extrapolate from one successful application to the next. *In vivo* NMR spectroscopy is shown to be informative when a xenobiotic perturbs metabolic pathways that are accessible to the technique, and it is useful for probing the partitioning of paramagnetic metal ions between the cytoplasm and the vacuole. The successful application of ^{19}F NMR to the analysis of plant tissue extracts also suggests that *in vivo* ^{19}F NMR spectroscopy may have a role in biotransformation studies of fluorinated xenobiotics. In contrast NMR imaging techniques have been little used for xenobiotic research in plants, and while the method has been shown to be capable of monitoring the uptake and translocation of paramagnetic ions in plants, the potential use of high resolution ^{1}H and ^{19}F NMR imaging for mapping agrochemicals in tissues is still in its infancy.

Abbreviations: ALS – acetolactate synthase; AMPA – aminomethylphosphonate; EPSP – 5-*enol*-pyruvoylshikimate 3-phosphate; NMR – nuclear magnetic resonance; P_i – inorganic phosphate; 2,4-D – 2,4-dichlorophenoxyacetic acid.

Introduction

The direct detection of nuclear magnetic resonance (NMR) signals from living systems has several well known advantages in comparison with analyses based on tissue extraction. First, by avoiding the necessity for tissue extraction and the subsequent work-up of the sample, *in vivo* NMR methods may be more convenient or more reliable than *in vitro* analyses. Secondly, because all the detectable compounds that exceed the detection threshold for an NMR experiment are necessarily observed simultaneously, there is no preselection by the investigator, with the result that *in vivo* NMR methods can reveal unexpected information that would otherwise escape detection. Thirdly, as a non-invasive technique, *in vivo* NMR may yield information that would be undetectable *in vitro*, such as information on the intracellular environment and the compartmentation of metabolites. Finally, *in vivo* NMR provides many opportunities for testing metabolic hypotheses directly in functioning systems, and this sheds light on the way in which metabolism is integrated *in vivo*. These advantages of *in vivo* NMR have to be set against the equally well known disadvantages, namely the relatively low sensitivity of the technique and the requirement for technologically advanced, and therefore expensive, equipment. However these disadvantages are not overwhelming and the result is that *in vivo* NMR methods are frequently deployed very effectively in biochemi-

cal and physiological investigations of living systems (Gadian, 1995).

The *in vivo* NMR approach is readily applied to plants and there is now an extensive literature describing the use of both NMR spectroscopy and NMR imaging in this field (Ratcliffe 1994, 1996; Shachar-Hill & Pfeffer 1996). Major areas of application for *in vivo* NMR spectroscopy in plants include: (i) nitrogen and phosphorus nutrition; (ii) the pathways of carbon and nitrogen metabolism, exploiting the potential of stable isotope labelling with ^{13}C and ^{15}N; (iii) metabolic responses to environmental stress, including the effects of anoxia, drought and salt stress; (iv) cytoplasmic and vacuolar pH regulation; and (v) ion transport and compartmentation. Similarly high resolution NMR imaging can now generate spatially resolved analytical information on the tissue distribution of water and other abundant plant constituents, as well as measurements of diffusion and flow (Chudek & Hunter 1997). The extensive use of *in vivo* NMR methods that has been made in the study of the response of plant tissues to abiotic factors, for example oxygen deprivation (Ratcliffe 1997), would seem to suggest that a similar approach could be useful in the study of the interaction of plants with xenobiotics. However applications of this kind are still rarely encountered and this review article explores the reasons for this lack of activity.

In principle, non-invasive NMR methods might be expected to find applications in three areas of xenobiotic research in plants. First, NMR methods might be used to characterise the effects of xenobiotics on the metabolism of susceptible and resistant plant species. Secondly, it might be possible to use the NMR approach for the direct detection of xenobiotics and their derivatives *in vivo*. Finally, it might be possible to obtain information about the spatial distribution of xenobiotics and their derivatives, either indirectly through spectroscopy or directly through NMR imaging. Taking xenobiotics to include both the plant protection products generated by the agrochemical industry and pollutants such as heavy metals, there would seem to be considerable scope for using *in vivo* NMR methods to complement conventional *in vitro* analyses. In fact the potential applications in plant xenobiotic research for *in vivo* NMR have been apparent for many years, and early attempts to look at the metabolism of agrochemicals and their metabolic effects by a combination of *in vitro* and *in vivo* NMR methods were discussed in a more general review on the NMR analysis of agrochemicals and pesticides (Bright & Ratcliffe 1990). The present review describes the progress that has occurred in the last few years in this field and discusses the extent to which *in vivo* NMR can have a problem solving role in xenobiotic research. It will become apparent that while *in vivo* NMR methods lack the generality that is usually associated with routine analytical tools, they can still provide novel information in particular cases.

Metabolic effects of xenobiotics

Metabolic inhibitors with known properties are frequently used in the course of *in vivo* NMR studies of metabolism, and so in principle it should be possible to use the same approach to characterise the metabolic disruption caused by the uptake of herbicides and other xenobiotics. Herbicides are usually designed to inhibit specific metabolic processes, and so the expectation would be that the NMR experiment might be able to identify some of the direct consequences of the activity of the compound. In practice the literature contains very few significant applications of *in vivo* NMR spectroscopy for this purpose, but the general approach can be illustrated by an investigation of the effect of glyphosate on the metabolism of *Acer pseudoplatanus* suspension cells (Gout et al. 1992). Glyphosate inhibits 5-*enol*-pyruvoylshikimate 3-phosphate (EPSP) synthase and thus prevents the synthesis of aromatic compounds via the shikimate pathway, including the synthesis of phenylalanine, tyrosine and tryptophan. A combination of ^{31}P NMR and natural abundance ^{13}C NMR was used to probe the metabolic effects of glyphosate *in vivo* and both approaches yielded novel information (Figures 1 and 2).

In vivo ^{31}P NMR (Figure 1) showed that exposure to 1 mM glyphosate had no obvious effect on the usual metabolic signature of the *Acer* cells, even though it caused a 90% inhibition of growth. This result immediately highlights the point that *in vivo* NMR only detects a subset of the important metabolites in a cell and these pools will not necessarily be sensitive to the xenobiotic treatment. However the ^{31}P NMR spectra also contained signals from glyphosate, aminomethylphosphonate (AMPA), which is a biodegradation product of glyphosate, and shikimate-3-phosphate, which is a substrate for EPSP synthase. The detection of the shikimate-3-phosphate signal, which was identified on the basis of parallel NMR analyses on cell extracts, was the only indication

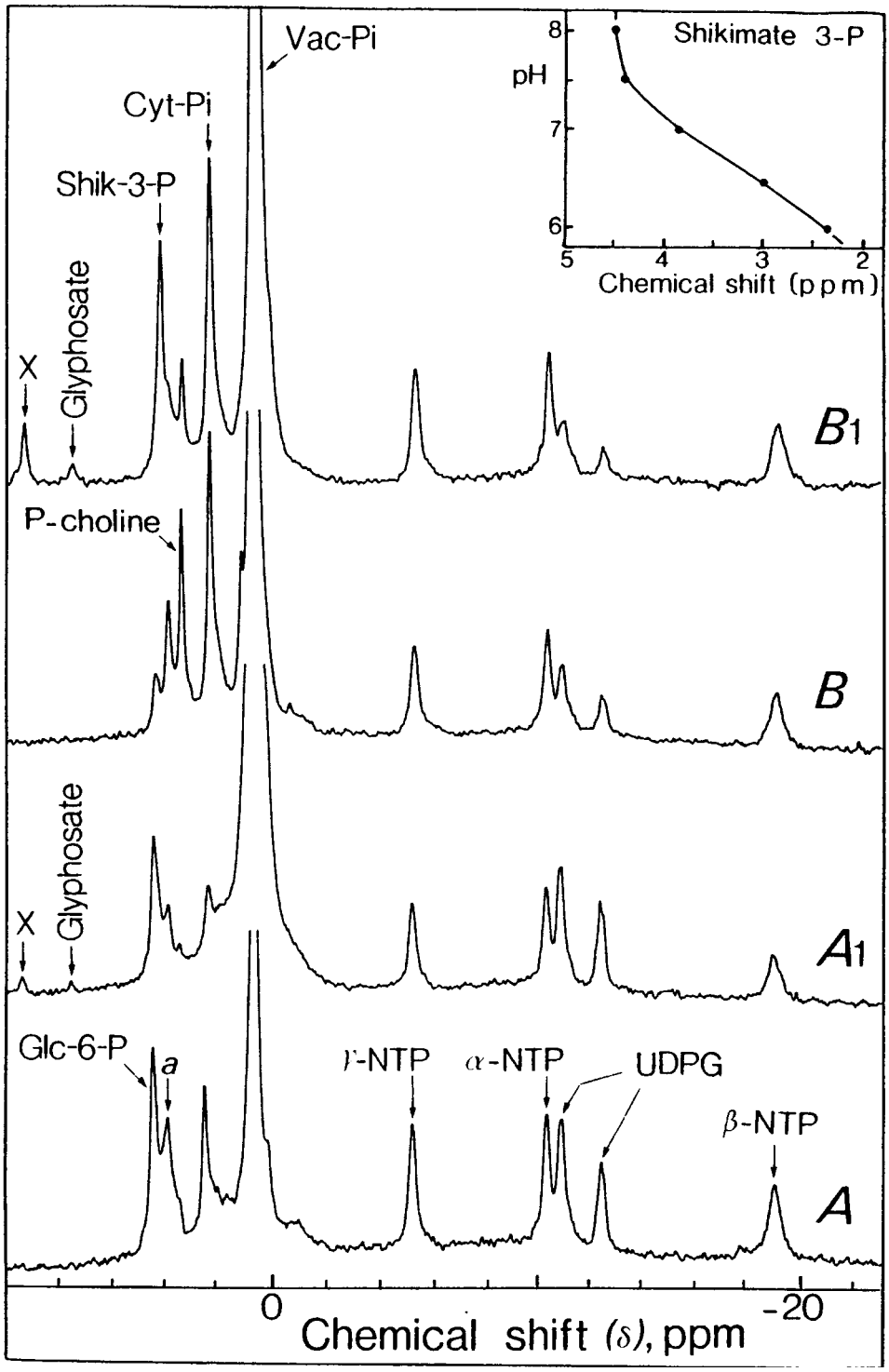

Figure 1. In vivo ^{31}P NMR spectra of sycamore cells showing the effect of a 50 h incubation in the following solutions: A, standard culture medium; A_1, standard culture medium plus 1 mM glyphosate; B, sucrose-free culture medium; B_1, sucrose free culture plus 1 mM glyphosate. Peak X can be assigned to AMPA and peak a is a composite signal from several phosphomonesters. The insert shows the pH dependence of the chemical shift of the shikimate 3-phosphate signal. Reprinted from Gout et al. (1992) with the permission of the Société Française de Biochimie et Biologie Moléculaire.

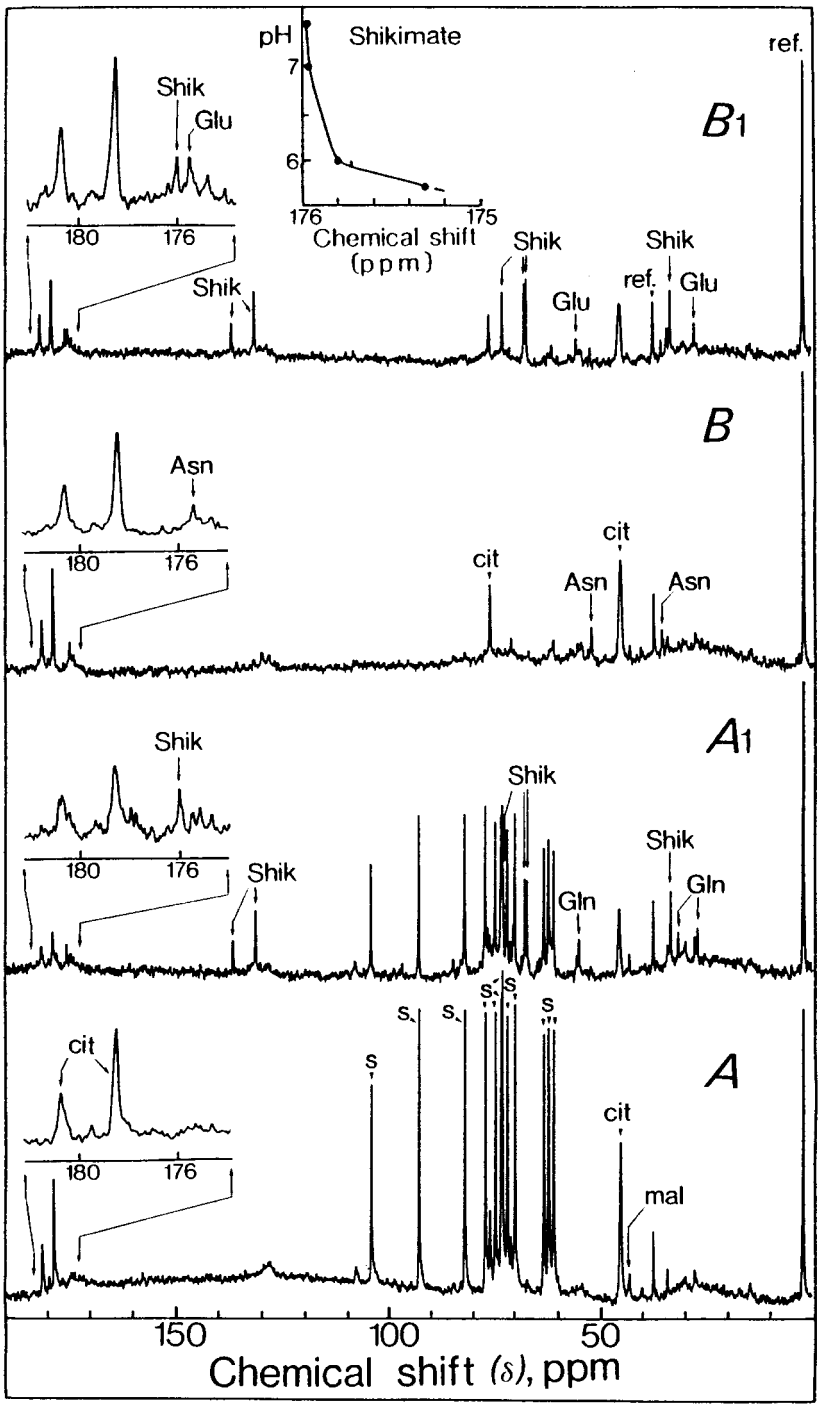

Figure 2. In vivo ^{13}C NMR spectra of sycamore cells incubated under the conditions defined in the legend to Figure 1. The peak assignments include: asn, asparagine; cit, citrate; glu, glutamate; mal, malate; s, sucrose; and shik, shikimate. The carboxyl region of the spectrum is shown on an expanded scale, and the insert shows the pH dependence of the shikimate carboxylate group. Reprinted from Gout et al. (1992) with the permission of the Société Française de Biochimie et Biologie Moléculaire.

of the metabolic disruption caused by glyphosate, and it provided a clear demonstration of the expected inhibition of EPSP synthase. A further interesting point was that the glyophosate, AMPA and shikimate-3-phosphate pools could all be shown to be cytoplasmic on the basis of the pH-dependent ^{31}P NMR chemical shift values. This information on the subcellular compartmentation of the molecules would have been difficult to obtain from tissue extracts and it therefore provides a good example of the usefulness of the *in vivo* approach.

The natural abundance ^{13}C NMR spectra of glyphosate treated *A. pseudoplatanus* cells were also informative (Figure 2). Firstly, the spectra demonstrated a very large accumulation of cytoplasmic shikimate, and when taken with the ^{31}P NMR data on the accumulation of shikimate-3-phosphate, it was concluded that neither metabolite could be acting as a feedback inhibitor on the early steps of the shikimate pathway. Secondly, the ^{13}C NMR spectra showed that there was a marked accumulation of glutamate and glutamine in the cells, and this was attributed to the inhibition of aromatic amino acid synthesis and the consequent halt in protein synthesis and growth. This observation illustrates the way in which NMR can generate interesting correlations between metabolic events by simultaneously providing data on a whole range of compounds and not just those that might have been expected to be of interest.

The overall conclusion from these experiments was that NMR spectroscopy could provide a powerful method for studying the metabolic impact of a herbicide (Gout et al. 1992). In fact the NMR analysis of the tissue extracts was at least as informative as the *in vivo* measurements in these experiments, and the main advantages of the latter can be seen to have been: (i) the novel information that was obtained from the *in vivo* spectra on the subcellular location of several compounds; (ii) the ease with which timecourses of the metabolic effects could be obtained over extended periods; and (iii) the validation of the results from the tissue extracts. Thus while much of the investigation could have been carried out at the level of tissue extracts, and while extracts were necessary to provide assignments for the *in vivo* spectra, NMR measurements on the cells themselves provided a convenient and informative way of probing the effects of the herbicide.

The balance between *in vitro* and *in vivo* NMR analyses moved in favour of tissue extracts in recent observations on the effect of sulfometuron methyl on the metabolism of the same sycamore suspension cultures (Aubert et al. 1997). Sulfometuron methyl is a sulphonyl urea herbicide that inhibits acetolactate synthase (ALS) and thus prevents the synthesis of branched chain amino acids. The herbicide also causes a marked increase in the activity of the alternative oxidase and in seeking to understand this, it was important to characterise the effect of sulfometuron methyl on the metabolism of the cells. *In vitro* NMR measurements showed that exposure to the herbicide led to the accumulation of α-oxobutyrate, which is a substrate of ALS, and α-aminobutyrate, which is a transamination product of α-oxobutyrate. It was also shown that supplying these metabolites in the absence of the herbicide led to the detection of the branched chain amino acid isoleucine; and ultimately it was concluded that the induction of the alternative oxidase by sulfometuron methyl was probably caused by the blockage of branched chain amino acid synthesis, since induction could be prevented by supplying leucine, valine and isoleucine in the presence of the inhibitor (Aubert et al. 1997). Thus in this study, *in vitro* ^{13}C NMR provided a convenient metabolic profiling technique for confirming that the expected effects of the herbicide had taken place and there was no compelling reason for undertaking measurements *in vivo*.

Intracellular pH values can also be obtained by *in vivo* NMR measurements and changes in the cytoplasmic and/or vacuolar pH values of plant cells have frequently been observed in response to abiotic stress. No such changes were observed in the glyphosate study, but some observations on the uptake of 2,4-dichlorophenoxyacetic acid (2,4-D) provide a recent example (Kasai & Bayer 1995). In this paper, maize root tips exposed to 200 μM 2,4-D showed a small, 0.25 pH unit acidification of the cytoplasm that was attributed to the accumulation of a cytoplasmic pool of the herbicide. These observations are relevant to questions concerning the mechanism of uptake of 2,4-D, rather than to its metabolic impact on the plant cell, but they provide another indication of the way in which an *in vivo* NMR experiment can add to current knowledge about the mode of action of herbicides.

Heavy metals can also have toxic effects on plants, but most of the *in vivo* NMR studies in this area have focused on the uptake and accumulation of the ions rather than on their metabolic effects. Many of these ions are paramagnetic, and while this can be exploited in studies of the subcellular compartmentation of heavy metals (see the section on the spatial locali-

sation of xenobiotics in plants), the same ions tend to degrade the quality of the spectra through line broadening, making it more difficult to resolve the signals and thus to detect any changes in the metabolic composition of the perturbed tissue. Accordingly, it seems likely that metabolic perturbations will only be seen when the paramagnetic effects are relatively modest or when the heavy metal is diamagnetic, as in the case of Zn^{2+}, Cd^{2+} or Pb^{2+}. For example, it would seem worthwhile to use NMR methods to follow up recent work on the hyperaccumulation of nickel by *Alyssum lesbiacum* (Krämer et al. 1996). Nickel hyperaccumulation depends on the ability of the root system to produce substantial amounts of histidine as a nickel complexing ligand in this species, and the metabolic adjustments that are necessary to cope with the influx of nickel could be explored using *in vivo* ^{15}N NMR (JAC Smith, pers. comm.).

Aluminium provides an example of a diamagnetic cation and some of the effects of aluminium uptake by maize roots have been investigated by *in vivo* ^{31}P NMR (Pfeffer et al. 1986, 1987). In fact aluminium is not strictly relevant to this review, since it is a source of abiotic rather than xenobiotic stress, and it is in any case not a heavy metal. However from an NMR perspective aluminium is interesting because the favourable NMR properties of the ^{27}Al nucleus allow it to be detected directly in tissues that accumulate the metal, including the tea plant (Nagata et al. 1993), the mycorrhizal fungus *Laccaria bicolor* (Martin et al. 1994), and the leaves of hydrangea (Ma et al. 1997). The Al^{3+} ion exists in a variety of complexed forms *in vivo* and this approach allows conclusions to be drawn about the identity of the complexes.

Biotransformation of xenobiotics

The magnetic isotopes that are likely to be most useful in the NMR detection of xenobiotics and their degradation products are ^{1}H, ^{13}C, ^{15}N, ^{19}F and ^{31}P. An immediate practical problem is that xenobiotics often exert their effects at concentrations that are lower than the detection threshold for NMR analysis. This puts severe limitations on the extent to which such compounds might be detected *in vivo*, and indeed NMR detection may only be possible by working with much higher concentrations than the plants would normally encounter. In contrast, tissue extracts can usually be concentrated to a suitable level for NMR analysis, and this permits a more physiologically relevant experiment, while losing the inherent advantages of direct detection.

^{1}H NMR has the advantage of high sensitivity, but the ubiquity of the hydrogen atom inevitably leads to considerable complexity in the spectra of tissues and their extracts. However two dimensional NMR techniques can provide an effective solution to this problem, and when combined with gas chromatography-mass spectrometry it becomes possible to analyse complex mixtures of plant origin (Fan 1996; Fan et al. 1997). In fact very little use has been made of this approach so far, either for characterising the metabolic effects of xenobiotics on plants or for detecting the metabolism of xenobiotics by plants, and this is in marked contrast to the routine use of ^{1}H NMR for the structural characterisation of purified degradation products (Bright & Ratcliffe 1990).

The carbon atom is also ubiquitous, but while the detection threshold for ^{1}H NMR *in vivo* is around 100 μM or lower, the limit for natural abundance ^{13}C NMR is more than two orders of magnitude higher. The actual concentration threshold depends on several factors, including the intrinsic sensitivity of the NMR nucleus, the operating strength of the NMR magnet and the time available for the accumulation of the spectrum. However a reasonable indication of the sensitivity of natural abundance ^{13}C NMR can be obtained from the spectra in Figure 2. These spectra, which were accumulated in 2 h, gave clearly defined signals from shikimate, corresponding to a concentration of 10–15 mM averaged over the sample, but no signals from glyphosate or AMPA, both of which were detected *in vivo* by ^{31}P NMR (Gout et al. 1992). It follows that ^{13}C NMR is only likely to be practicable for the detection of xenobiotics in tissue extracts or *in vivo* if the compound of interest is first labelled with ^{13}C. Such stable isotope labelling is commonly employed for *in vivo* NMR studies of tissue metabolism, but it appears to have been attempted only rarely with xenobiotics, one exception being an attempt to detect the metabolism of 2,4-D in carrot root tissue (Loughman et al. 1987). This approach is probably more likely to be attractive for the characterisation of new compounds, than for the routine analysis of commercial products, since the likelihood of obtaining significant information is greater with such compounds, and it is also possible to take advantage of the synthetic capabilities of the team that created the new compound.

^{19}F NMR is a much more promising analytical technique for probing xenobiotic metabolism, since

the sensitivity of the method is comparable to that of ^{1}H NMR and there are usually no background signals from naturally occurring fluorinated compounds. ^{19}F NMR also has the advantage of a much wider chemical shift range than ^{1}H NMR, and this reduces the number of overlapping signals in ^{19}F NMR spectra. These favourable properties are frequently exploited in studies of drug metabolism in animals (Malet-Martino & Martino 1989, 1992; Komoroski 1994), and there has been some interest in using an analogous approach to study the metabolism of fluorinated plant protection agents by plants. The main emphasis has been on the detection of fluorinated pesticide residues in crop plants (Mazzola et al. 1984; Mortimer & Dawson 1991b; Mortimer et al. 1994; Mabury & Crosby 1995), and it has been established that ^{19}F NMR provides a sensitive and convenient method for identifying a wide range of herbicides, fungicides and insecticides in plant tissue extracts. Concentrations as low as 1–5 μM can be readily detected in high field spectrometers, and in a typical application the trifluoromethyl group of trifluralin was detected in tissue extracts from carrots that had been grown in soil treated with the herbicide (Mortimer et al. 1994).

^{19}F NMR can also be used to detect the biodegradation of xenobiotics, and this approach has recently been used to investigate the metabolism of a fluorinated fungicide by *A. pseudoplatanus* cells (Serre et al. 1997). The interest lay in detecting and identifying the breakdown products of the fungicide, rather than in determining the time course of its metabolism, and so an inherently more sensitive and flexible *in vitro* analysis was used rather than attempting to detect the signals *in vivo*. Figure 3 shows spectra from hexane and ether fractions derived from the culture medium after six days incubation in the presence of the fungicide, and it can be seen that signals were readily detected from three derivatives of the original compound. A notable feature of this work was the way in which chemical changes at sites remote from the reporter trifluoromethyl group gave rise to small but detectable chemical shift differences in the ^{19}F NMR spectrum. The combination of a low concentration threshold for detection and the high sensitivity of the chemical shift to changes in molecular structure is particularly advantageous, and it was concluded that ^{19}F NMR was a powerful method for detecting the biotransformation of fluorinated xenobiotics by plant cells.

The only other NMR nucleus that has found some application in the detection of xenobiotics and their degradation products in plants is ^{31}P. For example it has been established that organophosphorus insecticides can be detected at the 0.5 ppm level in acetone extracts of 50 g of broccoli or cabbage using only 30 min of NMR time at 400 MHz (Mortimer & Dawson 1991a). *In vivo* applications of ^{31}P NMR are also possible, and Figure 1 shows that both glyphosate and its breakdown product AMPA were readily detectable in sycamore cells after an incubation period of 50 h. As in the case of ^{19}F NMR, ^{31}P NMR benefits from the relative simplicity of the spectrum, and in the absence of readily available [^{13}C]labelled xenobiotics it would appear that it is ^{19}F and ^{31}P NMR, rather than ^{1}H and ^{13}C NMR, that are the most promising NMR techniques for the routine analysis of xenobiotic metabolism in plants.

Spatial localisation of xenobiotics in plants

In vivo NMR offers two fundamentally different approaches for obtaining spatial information from living systems. Firstly, the chemical shift and/or relaxation properties of an NMR signal may be inherently sensitive to the spatial location of the magnetic isotope, enabling spatial information to be deduced from spectra recorded from spatially heterogeneous systems (Belton & Ratcliffe 1985). This approach can provide important information on the subcellular compartmentation of ions and metabolites, and in plants it can lead to the direct detection of signals from the cytoplasm and the vacuole. Secondly, spatial information can be encoded in an NMR signal by imposing magnet field gradients on the sample during the NMR experiment. This is the principle of NMR imaging, and in plants this leads to spatial information at the cellular level and above (Ratcliffe 1994; Chudek & Hunter 1997).

The *in vivo* NMR analysis of the subcellular distribution of an ion or metabolite is easiest when pools of the metabolite in different compartments give rise to NMR signals with different chemical shifts. Differences in pH between compartments can lead to resolvable signals if the NMR spectrum of a metabolite is pH-dependent, and this has frequently been exploited in plant tissues since there is usually a large pH difference between the cytoplasm and the vacuole. These effects are usually observed in ^{13}C and ^{31}P NMR spectra and several examples have already been noted in Figures 1 and 2.

Signals that can be assigned to particular subcellular compartments can also be used as probes for the

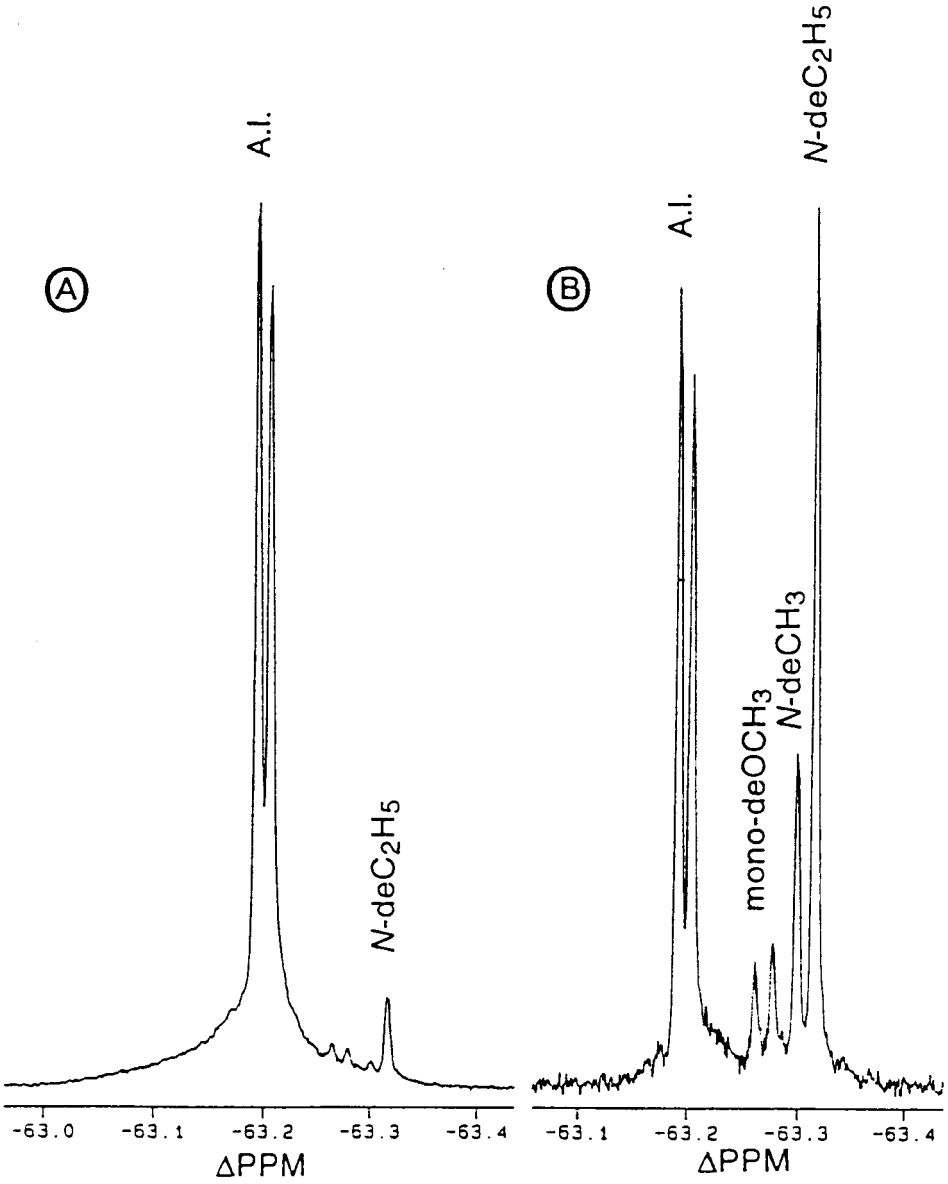

Figure 3. ^{19}F NMR spectra of the hexane (A) and diethylether (B) fractions originating from the medium of a sycamore cell suspension that had been incubated in the presence of the fluorinated fungicide *N*-ethyl-*N*-methyl-4-(trifluoromethyl)–2-(3,4-di methoxyphenyl)-benzamide. Signals were detected from the fungicide itself (A.I.), as well as from *N*-demethylated (*N*-deCH$_3$), *N*-deethylated (*N*-deC$_2$H$_5$) and monodemethoxylated (mono-deOCH$_3$) derivatives. Reprinted from Serre et al. (1997) with the permission of the American Chemical Society.

subcellular distribution of paramagnetic ions derived from the transition metals and the lanthanides. The basis of these experiments is that interactions between a paramagnetic ion and an NMR detectable ligand usually lead to changes in the position, linewidth and relaxation times of the ligand signals. These effects can be monitored *in vivo*, and they can then be used to detect the uptake and localisation of heavy metals. Changes in relaxation and lineshape have been exploited more often than changes in chemical shift, and the most useful paramagnetic ions for this purpose are probably Mn^{2+} and Gd^{3+}, since they often cause changes in relaxation without any effect on the chemical shift.

Figure 4 illustrates the approach with a series of ^{31}P NMR spectra recorded from maize root segments

during exposure to gadolinium (Quiquampoix et al. 1990). The detection of separate signals from the cytoplasmic and vacuolar inorganic phosphate (P_i) pools allows the presence of gadolinium to be detected in both compartments and indeed this experiment provided the first direct evidence that a lanthanide, in this case gadolinium, could accumulate as a soluble pool within a plant cell (Quiquampoix et al. 1990). The same approach has been used to investigate the uptake and localisation of manganese by maize root tissues (Kime et al. 1982; Pfeffer et al. 1986; Quiquampoix et al. 1993a, 1993b) and *A. pseudoplatanus* cell suspensions (Roby et al. 1988). The extent to which soluble Mn^{2+} is partitioned between the cytoplasm and the vacuole can be judged from the changes in the cytoplasmic and vacuolar P_i signals, and this leads to good quantitative evidence for the role of the vacuole as a sink for Mn^{2+} (Quiquampoix et al. 1993b). The polyphosphate signal in the ^{31}P NMR spectra of algae has also been used as a reporter for the presence of paramagnetic ions, and in a recent study this approach was used to detect the uptake of Mn^{2+}, Fe^{2+}, Co^{2+} and Cu^{2+} by *Stichococcus bacillaris* (Zhang & Majidi 1994).

Thus subcellular information can be deduced from an analysis of the *in vivo* NMR spectrum whenever a xenobiotic or its degradation product has a pH-dependent chemical shift in the pH range encompassed by the cytoplasm and the vacuole, or whenever a xenobiotic interacts with a detectable metabolite that can be assigned to the cytoplasm or the vacuole. These conditions are quite stringent, with the result that only a rather small number of plant protection agents are likely to satisfy them, but the method has been shown to be very effective in favourable cases. Thus glyphosate and its breakdown product accumulate to detectable, millimolar levels in plant tissues and have pH-dependent ^{31}P NMR signals (Figure 1); while paramagnetic ions such as Mn^{2+} and Gd^{3+}, at concentrations as low as micromolar, can influence the NMR signals of suitable ligands (Figure 4). In passing, it may be noted that *in vivo* ^{19}F NMR may also be useful for subcellular analysis, because of the sensitivity of the ^{19}F nucleus to its chemical environment, but the potential of this approach has yet to be explored in plant tissues.

NMR imaging generates spatial information in a rather different way from *in vivo* NMR spectroscopy, but the potential applications to the localisation of xenobiotics depend on strategies that are entirely analogous to those employed in spectroscopic studies. Thus if a xenobiotic, or a degradation product, were to accumulate to detectable levels in a plant tissue, then it would be possible to employ chemical shift imaging to generate a three dimensional distribution map. Alternatively if the xenobiotic were to affect the properties of some readily imaged component of the plant, for example the tissue water, then it would again be possible to deduce information about the spatial distribution of the compound of interest.

The potential of the chemical shift imaging approach can be judged from recent work on the distribution of sucrose and other metabolites in castor bean seedlings (Metzler et al. 1994, 1995; Ziegler et al. 1996), and from a paper reporting the detection of sugars in germinating barley seedlings (Ishida et al. 1996). The concentration threshold for chemical shift imaging is substantially higher than for *in vivo* NMR spectroscopy, reflecting the fact that the imaging experiment breaks down the signal from the whole sample into a set of smaller signals corresponding to voxels defined by the magnetic field gradients. There is an inverse relation between the size of the voxel and the minimum concentration for detecting a signal, but to obtain worthwhile spatial resolution it is currently necessary for the metabolites to be present at concentrations of at least a few millimolar. Thus in the case of the castor bean seedlings, working with a high field 11.7 Tesla magnet, it was estimated that the concentration threshold for detecting a signal in a voxel with a volume of 560 nl was of the order of 10 mM for a data acquisition time of 4 h 33 min (Metzler et al. 1995). These figures are indicative of what can be achieved with state of the art technology, but it should be noted that the actual value for the concentration threshold is always going to be a function of the sample, the available instrumentation and the parameters of the radio frequency coil that is used to generate the image.

^{1}H chemical shift imaging has not yet been used for the detection of xenobiotics in plants, but fluorine imaging, which has a comparable sensitivity and is easier to implement because of the absence of background signals, has been used in exploratory studies (Rollins et al. 1989). The uptake of trifluoracetic acid was monitored in tomato plants in these experiments, and the fluorine images showed the presence of the xenobiotic in the vascular tissues of the stem. While these observations give little indication of what might be possible with a modern high field imaging system, it is clear that the approach will be limited to compounds that can reach at least millimolar concentrations in the tissue and this would suggest, given the

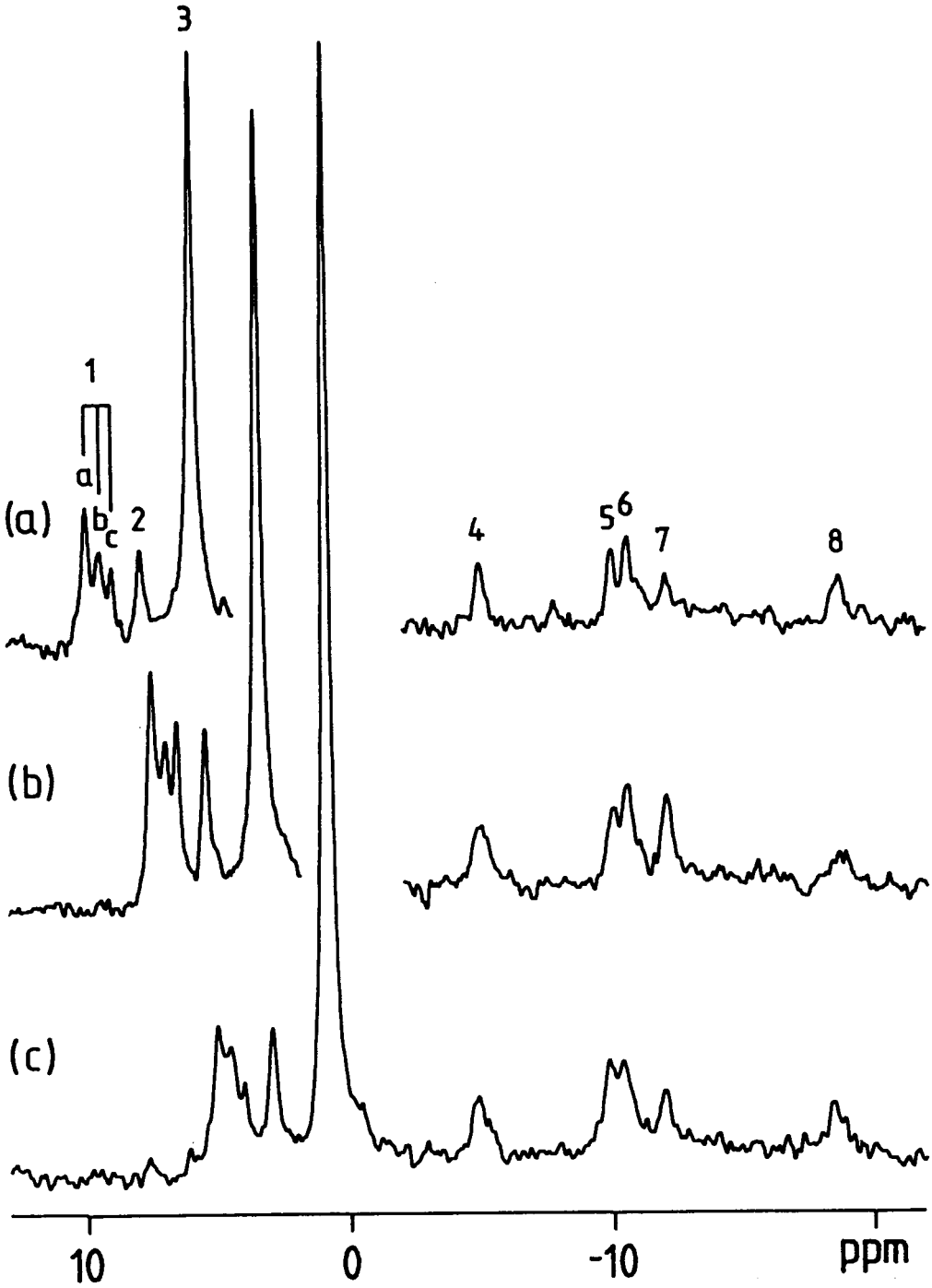

Figure 4. In vivo ^{31}P NMR spectra of maize root segments showing the effect of gadolinium uptake into the cytoplasm and vacuole. After recording the initial spectrum (a), 100 μM Gd^{3+} was added to the suspending medium and spectra were recorded after (b) 2.25 h and (c) 9.25 h exposure to gadolinium. The spectra were recorded under conditions that caused partial saturation of peaks 1–3, and the increase in the peak heights of these signals in (b) indicates a paramagnetic contribution to the relaxation rates as a result of the influx of soluble Gd^{3+} into the cytoplasm and vacuole. The peak assignments are: 1, several phosphomonesters, including glucose 6-phosphate (1a) and phosphocholine (1c); 2, cytoplasmic P_i; 3, vacuolar P_i; 4, 5 and 8, the γ-, α- and β-phosphates respectively of nucleoside triphosphate (NTP); 6, UDP-glucose and NAD(P)(H); and 7, UDP-glucose. Adapted from Quiquampoix et al. (1990) with the permission of Elsevier Science.

potency of many modern agrochemicals, that the potential of the technique lies in probing the distribution and transport of fluorinated compounds in resistant species.

An alternative NMR imaging approach would be to deduce the spatial distribution of the xenobiotic from its effect on the tissue water image. High spatial resolution water images can be obtained in a timescale of minutes, for example Metzler et al. (1994) recorded an image corresponding to an in-plane resolution of $24 \times 24 \mu m$ and a slice thickness of 1 mm in 32 min, and the contrast in such images is sensitive to the presence of paramagnetic ions. It follows that the uptake and translocation of paramagnetic heavy metals can be studied directly on intact seedlings, and the practicality of the method has been demonstrated for both Cu^{2+} (Bottomley et al. 1986) and Mn^{2+} (Connelly et al. 1987; Koizumi et al. 1992). The underlying reason for the change in contrast is the paramagnetic contribution to the relaxation times of the water magnetisation, and in principle it should be possible to quantify this effect using multi-echo T_2 imaging sequences. This approach has been used to assess the level of iron in the human liver (Papakonstantinou et al. 1995), and it has also been applied to plants (Edzes et al. 1998), although not as yet for the quantitative analysis of paramagnetic ions. Despite this, it is clear that it is much easier to adapt the NMR imaging technique to the detection of paramagnetic ions than it is to detect a xenobiotic with plant protection properties.

Conclusion

Several conclusions emerge from this review of the way in which *in vivo* NMR methods can be used in xenobiotic research in plants. First, *in vivo* NMR methods have been used rather infrequently in xenobiotic studies on plants, and this probably reflects a degree of unpredictability in the approach. Thus the successful application of an *in vivo* NMR experiment to one xenobiotic does not automatically lead to a routine method that will be successful with a whole family of related compounds. Secondly, exposure to xenobiotics does not necessarily lead to the detection of interesting spectroscopic changes because many agrochemicals will target pathways that are unrelated to the metabolic processes that can be observed by NMR. Thirdly, NMR analysis of tissue extracts is likely to be preferable to the *in vivo* detection of xenobiotic metabolism on grounds of sensitivity, but the most obvious candidate for a more detailed appraisal of *in vivo* methods in this area would be ^{19}F NMR. Finally, paramagnetic metal ions have predictable effects on the NMR properties of their ligands, and this favours the use of both *in vivo* NMR spectroscopy and NMR imaging for determining the spatial distribution of certain heavy metals in plants.

The overall conclusion is that *in vivo* NMR methods can generate novel information relevant to xenobiotic reseach and that such methods can complement the results obtained from the analysis of tissue extracts in several areas. Moreover, while *in vivo* NMR is far from being an indispensable tool in xenobiotic research, its usefulness will undoubtedly increase with the continuing development of NMR methods and hardware.

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