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Abstract

Using a new method of "susceptibility matching" by CCl4, we found that the proton nuclear magnetic resonance (NMR) spectrum of intact mustard (Sinapis alba L.) seeds consists of NMR lines, originating from oil bodies (microdroplets) surrounded by a solid structure. The intact seed resonances in CCl4 were identified as CH3 at 0.9 ppm, -(CH2)n- at 1.3 ppm, and -CH=CH- at 5.3 ppm, respectively. The protons of the glyceride structure were detectable at 4.2 ppm. An additional heterogeneous line was also measured at about 2.2 ppm.

Reference


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METHODS

Nuclear Magnetic Resonance Study of Lipids in Mustard Seeds

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Abstract – Using a new method of “susceptibility matching” by CCl₄, we found that the proton nuclear magnetic resonance (NMR) spectrum of intact mustard (Sinapis alba L.) seeds consists of NMR lines, originating from oil bodies (microdroplets) surrounded by a solid structure. The intact seed resonances in CCl₄ were identified as –CH₃ at 0.9 ppm, -(CH₂)n – at 1.3 ppm, and –CH=CH– at 5.3 ppm, respectively. The protons of the glyceride structure were detectable at 4.2 ppm. An additional heterogenous line was also measured at about 2.2 ppm.

Key words: Sinapis alba - nuclear magnetic resonance - spin-lattice relaxation - susceptibility matching - oil bodies

INTRODUCTION

NMR in steady-state and in the pulse regime has proved to be a powerful tool for noninvasive investigation of mobile components in plant tissues (leaves, seeds, roots, pulps, or bulbs) [1 - 9].

In our previous work, we studied the mobility of water molecules in the seeds of oat and barley during germination using NMR [7, 8]. The dry oat and barley seeds contain a small amount of mobile molecules. Consequently, the NMR spectral lines are broad, the relaxation times are very short, and frequently the relaxation decays are not detectable.

The mustard seeds belong to the category of oil-containing plant systems, where the fast component of molecular dynamics is given by the motion of oil molecules.

MATERIALS AND METHODS

Intact mustard seeds (Sinapis alba L.), 2.1 ± 0.2 mm in diameter were directly introduced into the NMR 5-mm standard tubes. The NMR spectra were measured on Hitachi-Perkin-Elmer 24 A (60 MHz) and Varian XL-200 (200 MHz) spectrometers.

For spectroscopic reasons, we have elaborated another approach for susceptibility matching in the samples studied.

This problem, discussed by Zimmerman and Forster [10], given by the fact that the susceptibility differences between the seeds and their environment (air) contribute significantly to the increase in the nonhomogeneity of the magnetic field inside the sample. Consequently, this effect broadens the NMR spectral line. Conway and Johnson [2] attempted to reduce the susceptibility differences by drying corn kernels to 4% moisture and by subsequent soaking in Freon-113 for 6 days. From this, they obtained an adequate resolution and a good signal-to-noise ratio.

In our case, we attempted to reduce the gradient of susceptibilities by filling the space between seeds with some liquids that do not contain protons (Fig. 1). The application of tetrachloromethane (CCl₄) gives better results than hexafluorobenzene (C₆F₆).

In order to interpret the results, we also measured the NMR spectrum of oil extracted from the mustard seeds with petroleum ether and of the remaining powder. According to Stefanov et al. [11], oil in mustard seeds is essentially composed of glycerides with the following fatty acid composition: erucic acid, oleic acid, and linoleic acid. We thus identified the resonances of the extracted oil by comparing them with the spectra of these authentic pure substances and a pure triglyceride (triolein) at 200 MHz.

The spin-lattice and spin-spin relaxation times were measured on a home-made relaxometer working at 25 MHz [9]. The probe used was of the crossed coil

Abbreviation: NMR – nuclear magnetic resonance

Fig. 1. The principle of “susceptibility matching” by the liquid CCl₄, in which the mustard seeds (G) were submerged.
type, situated in the 24-mm gap of a permanent magnet. The maximum power of the transmitter during the RF pulses was 20 W into a 50-Ohm load. The transmitter coil was made up of two sections (Helmholtz system), one on each side of the sample. Each section was rectangular in shape. The receiver coil was cylindrical and 4 mm long. NMR tubes of diameter 5, 7, or 8 mm can be used.

The spin-lattice relaxation times were obtained by the well-known "inversion-recovery" sequence and the spin-spin relaxation times were obtained by the Carr-Purcell-Gill-Meiboom pulse sequence [9].

RESULTS

The water content of the mustard seeds measured by the gravimetric method was 6% of fr wt. The oil content was 24%.

The proton NMR spectra at 60 MHz are presented in Fig. 2. Spectrum A was found in the intact mustard seeds, B is given by the seeds submerged in CCl₄, and C is the spectrum of oil extracted from the mustard seeds.

A comparison of these results shows the similarity of these spectra A, B, and C, despite the difference in their line width. In spectrum B, in which we applied the CCl₄, the spectral lines are narrower than in A.

The transition between spectra A and B is reversible. This means that after elimination of CCl₄, the mustard seeds again yield spectrum A, and so on.

In spectrum C, one can identify the main lines at 0.9 ppm, 1.33 ppm, 2.2 ppm, 2.8 ppm, 4.2 ppm and a multiplet, centered at 5.3 ppm.

The spectrum of the mustard oil extract in CDCl₃ (deuterated chloroform) at 200 MHz is presented in Fig. 3. We can thus assign the main resonances of the intact mustard seeds in CCl₄ (Fig. 2B) as -CH₃ at 0.9 ppm, -(CH₂)ₙ– at 1.3 ppm and -CH=CH– at 5.3 ppm, respectively. The protons of the glyceride structure were detectable at 4.2 ppm. An additional heterogeneous line was also measured at about 2.2 ppm.
The NMR spectra of mustard seeds do not exhibit any anisotropy. The dehydrated seeds yield the same spectra as the nondehydrated ones. The resulting powder, from which the oil was extracted, does not yield any NMR spectrum. Domestic oil yields a NMR spectrum very similar to that presented in Fig. 2C.

The values of the spin relaxation times, $T_1$ and $T_2$, are presented in the table.

### DISCUSSION

A comparison of the NMR spectra presented in Fig. 2 shows that the spectrum of the intact mustard seeds is entirely determined by the protons of the mobile components of oil. This is confirmed by the fact that the remaining powder, after extraction of the mustard oil, does not exhibit any NMR spectrum, whereas the entire NMR spectrum is present in the extracted oil.

Surprisingly enough, there is only a small difference between the molecular dynamics in the rigid seed and in the extracted oil, because the ratio of the corresponding spin-lattice relaxation times is only 0.902. This means that the broadening of the NMR lines is given mainly by the susceptibility gradients in the sample.

Why are the molecular dynamics modified so little by the rigid structure of the seed? In the following model, presented by Huang [12], the oil in the seed is organized in so-called “oil bodies”. This is a very well-organized spherical molecular aggregate, 0.2 - 2.5 μm in diameter. Oil bodies of mature seeds, either inside the cell or as isolated preparations, maintain a hydrophilic surface. They are remarkably stable and do not aggregate or coalesce. Two factors are found to contribute to the stability of the oil bodies: a) steric hindrance and b) negatively charged surface.

The surface oleosins, phospholipids, and a small quantity of free fatty acids interact to produce a cohesive and stable barrier, limiting the space of the oil body. Oleosins are alkaline proteins of low mol wt (15 - 26 kD). They are unique to oil bodies, as indicated by the subcellular fractionation and immunocytochemistry [13].

The internal space of an oil body is sufficiently large to allow the majority of the oil components (triglycerides) to exhibit the same Brownian motion as in the extracted oil sample. Only a small portion of lipids, situated near the interface limiting the shape of the oil bodies, will have their molecular movement more restricted.

As the passage between spectra A and B (Fig. 2) is reversible, it seems that the action of CCl₄ is rather limited at the surface of the mustard seeds. This is confirmed by our observation that CCl₄ that has been in contact with the seeds for a long time (2 days), does not show any measurable spectrum in our conditions when measured alone.

Further, there is no spectral line of water in the NMR spectra of mustard seeds. This was confirmed by the measurement of the dehydrated seeds. Note that the water concentration is small and the water dynamics is restricted.

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### REFERENCES


