Nuclear magnetic resonance microscopy of Ancistrocladus heyneanus

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Summary. The tropical liana Ancistrocladus heyneanus, which is known for its biologically active naphthylisoquinoline alkaloids, has been studied by nuclear magnetic resonance (NMR) microscopy for the first time. The spatial resolution of the cross-sectional NMR images was of the order of 20 μm. Quantitative NMR relaxation time images of the root and the shoot show great contrast between different tissue regions. In addition, we observed the regional distribution of chemical compounds in Ancistrocladus heyneanus by chemical-shift NMR microscopy. The NMR imaging results were compared with light and fluorescence microscopic images and reveal the excellent tissue characterization using NMR technology.

Keywords: Nuclear magnetic resonance micro-imaging; Chemical-shift imaging; Fluorescence microscopy; Ancistrocladus heyneanus; Ancistrocladaceae; Naphthylisoquinoline alkaloids.

Abbreviations: NMR nuclear magnetic resonance; CSI chemical-shift magnetic resonance imaging; FOV field of view; TE echo time; TR repetition time.

Introduction

Plant families Ancistrocladaceae and Dioncophyllaceae are in many respects unique. They produce naphthylisoquinoline alkaloids (Bringmann and Pokorný 1995), natural substances not found in any other plants. This class of natural biaryls is characterized by its unusual molecular structure of a chiral biaryl axis, by unparalleled biosynthesis of isoquinoline alkaloids from acetic acid units, and by its promising pharmacological and other biological properties. Typical examples of these compounds are (1) ancistrocladine and (2) ancistroheynine A (Bringmann et al. 1996), which were isolated from Ancistrocladus heyneanus, as well as the dimeric alkaloid michellamine B (3), a strongly anti-HIV active substance, which is currently under preclinical investigation in the U.S.A. The chemical structures of these compounds are shown in Fig. 1.

The purpose of this work was to apply nuclear magnetic resonance technology to investigate the anatomy and biochemistry of Ancistrocladus heyneanus. NMR can be used to observe and localize the biosynthesis of the alkaloids and to identify the molecular structure and chemical state of these compounds in vivo.

Nuclear magnetic resonance (NMR) microscopy is a noninvasive and nondestructive method of examining the anatomy, function, and metabolism of intact plants (Bottomley et al. 1986; Connelly et al. 1987; Kuchenbrod et al. 1996, 1997; Metzler et al. 1994). It has been shown that the plant tissue structure can be observed with high image contrast and high spatial resolution. Spatial resolution can be as fine as a few micrometres. The NMR image contrast is freely variable and usually different to that of conventional light microscopy (Johnson et al. 1987). The physical basis of NMR microscopy is the localised observation of the ¹H NMR signal of water in the tissue (Callaghan 1991). Under certain experimental conditions the
Intrinsic NMR parameters, such as the NMR relaxation times $T_1$ and $T_2$, and nuclear spin density are influenced by a variety of chemical and physical parameters. The number of possible applications of NMR to plants is very large (Bentrup 1996). The image contrast depends on the spin density, the relaxation times $T_1$ and $T_2$, the diffusion coefficient of water, and the flow velocities in vessels (Kuchenbrod et al. 1995). In addition, biochemical information can be obtained using chemical shift NMR imaging, a technology which combines high resolution NMR spectroscopy with imaging (Sarafis et al. 1990, Metzler et al. 1995, Rumpel and Pope 1992).

Most publications describe NMR imaging experiments on whole plants or excised plant tissues (Bottomley et al. 1986; Connelly et al. 1987; Johnson et al. 1987; MacFall et al. 1990, 1992). These papers document only a few possible NMR experiments and, therefore, only investigate limited aspects of plant physiology or biochemistry. This work presents an NMR investigation of Ancistrocladus heyneanus, including quantitative NMR microscopy and chemical-shift imaging. In order to support our findings, we used light and fluorescence microscopy for comparison.

Plant families Ancistrocladaceae and Dioncophyllaceae are very small, consisting of only 25 and 3 species, respectively. For the first in vivo NMR studies, we chose the Indian species Ancistrocladus heyneanus, since conditions for the successful cultivation of this plant under greenhouse conditions in the Botanical Garden of the University of Würzburg have recently been developed and thus intact plant material was available.

**Material and methods**

Plants were grown from mature seeds in a tropical greenhouse of the botanical garden of the University of Würzburg. Detailed information on the cultivation conditions has been given by Bringmann et al. (1991, Bringmann 1993). For NMR studies, fresh material of approximately two-year-old plants was used immediately after cutting. In the NMR spectrometer, the excised part of the plant was immersed in a water-filled glass tube. NMR images were taken from plant material outside the water. If space was not available inside the NMR magnet, 5–6 cm long sections of stem were used, sealed at the ends to prevent loss of water.

NMR microscopy has been described in detail elsewhere (Callaghan 1991). The experiments were performed using a Bruker AMX-500 NMR spectrometer with a magnetic field strength of 11.75 $T$. We applied actively shielded magnetic field gradient coils with a maximum gradient strength of 700 mT/m for NMR imaging. A Helmholtz-type radio frequency coil with a diameter of 5 mm, tuned to the $^1$H NMR resonance frequency of 500 MHz was used for the excitation and detection of NMR signals.

We used a two-dimensional cross-sectional NMR spin-echo imaging sequence. The image matrix sizes were $128 \times 128$ and $256 \times 256$ picture elements. The spatial resolution within the plane of the image was $18–38 \mu m$, and the slice thickness was $460 \mu m$. The quantitative $T_1$ NMR images were measured by a saturation recovery technique (Freeman and Hill 1971). For this purpose, sixteen images with recovery times $TR$ between 100 ms and 15 s were acquired and the $T_1$ image was calculated on a pixel-by-pixel basis. $T_2$ images were measured by magnetization prepared NMR microscopy (Haase et al. 1993). A series of sixteen images with different echo times was acquired to calculate a $T_2$ image. The total measuring time for a single NMR microscopy image was between 30 s and 30 min, depending on the experimental parameters.

An $^1$H chemical shift imaging (CSI) technique (Brown et al. 1982) with water suppression was used to measure the spatial distribution of metabolites within the plant. The slice thickness of the cross-sectional plane was $2 \ mm$, the image matrix was $64 \times 64$ picture elements and the spatial resolution $0.21 \ mm$. Each image element