Tetrahedron Letters No. 51, pp 4757-4760, 1976. Pergamon Press. Printed in Great Britain.

^{13}C NUCLEAR MAGNETIC RESONANCE SPECTRUM OF DRIED STAR ANISE FRUITS AND ITS HISTOLOGICAL IMPLICATIONS

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(Received in Japan 12 October 1976; received in UK for publication 2 November 1976)

We recently have shown that the states of cytoplasmic aucubin and sucrose in *Aucuba japonica* could be studied conveniently by a direct measurement of the ^{13}C nmr spectra of single intact seeds.[1] As this approach, which requires no separation procedures at all, seems to afford a promising tool for various phytochemical problems, we currently are doing a series of explorative experiments to assess the feasibility of high resolution nmr techniques for various plant tissues containing many classes of compounds. In this communication, we shall point out even histological information could sometimes be obtained by nmr of plant tissues.

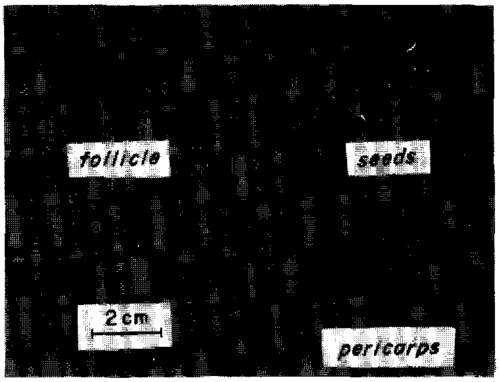

Figure 1. *Star anise*, dried follicles of *Illicium verum Hooker filius* produced in Peoples' Republic of China.

A dried fruit (follicles) of *star anise* (*Illicium verum Hooker filius*), which consists of seeds and pericarps (Figure 1), is a frequently used spice in the Chinese cuisine. A characteristic smell of the dried fruits is ascribed to the essential oil anethole. As single follicles can be split easily in halves by hands giving a pair of pericarps and a seed, we have searched for anethole in them separately.

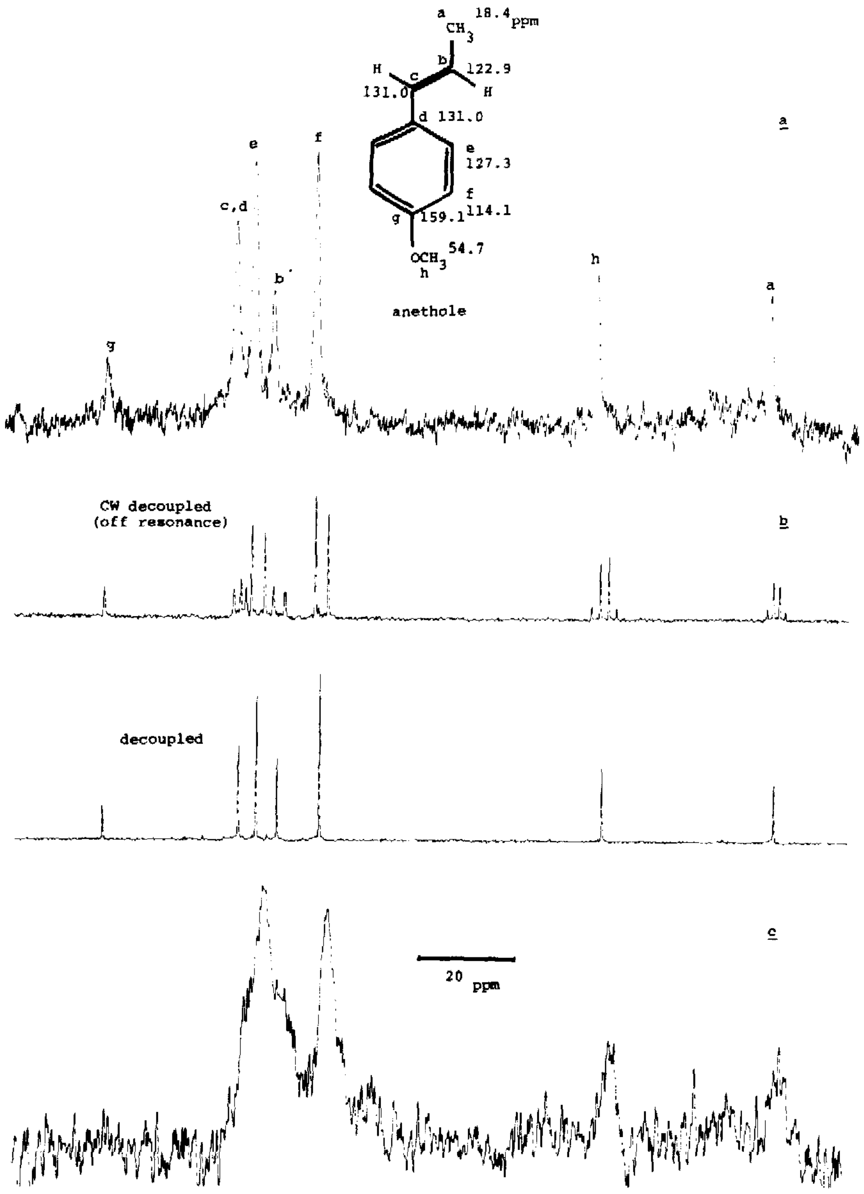

Figure 2. Proton decoupled ^{13}C Fourier transformed spectra of anethole contained in star anise pericarps. The spectra were run on a Varian XL-100 spectrometer at 25.2 MHz and at room temperature. The 12 mm sample tubes contained solid samples were spun usually at 70-80 rps. Chemical shifts of anethole are relative to the external tetramethylsilane (TMS) in the other sample tube. (a) Half-pericarps of star anise. (b) Neat anethole. (c) Powdered pericarps.

The ^{13}C nmr spectrum of anise seeds was very similar to those which had been found for soybeans and raddish seeds by Schaefer and Stejskal.[2] The spectrum therefore can be attributed to triglycerides contained in the seeds and the composition of fatty acid chains of the triglycerides may be determined from the spectrum[2]. Anethole, however, was not detected in the seeds. The pericarps, on the other hand, gave no triglyceride peaks and showed a set of resonances which were all assignable to anethole instead (Figure 2a). For a comparison, the ^{13}C nmr spectra of free anethole (a neat liquid) is shown in Figure 2b. Obviously there exists very little or no chemical shift differences between the spectra of anethole in the pericarps and in a neat liquid. This fact implies the anethole in pericarps is in a liquid state and bears little motional restriction. As the spectrum shown in Figure 2a was obtained for the uncrashed half-pericarps and about four to five of them were able to be accommodated in a 12 mm tube, the filling factor was far from the best. We then crashed the pericarps into fine powder using a pestle and mortar, in order to get a better spectrum. The powdered one, which of course could be packed tightly, gave an unexpected result (Figure 2c). A marked line broadening was evident for each of the resonances, implying that <u>the motional states of anethole became more restricted after the pericarps were mechanically crashed</u>. The result, which seemed for us rather surprising, was found to be very reproducible. We have explained this remarkable effect by considering the locality and states of anethole in the tissues.

In the uncrashed pericarps, anethole may be localized as oil drops in the parenchyma cells and therefore is in a motional state close to that of neat liquid. By crashing the pericarps, anethole undobtedly would leak from the cells and absorbs itself onto the powdered woody tissue uniformly. The motional state of anethole then would be more restricted under this circumstance. This is a clear example to show nmr can afford histological information for plant metabolites in tissues in some cases.

We studied also a dried <u>nutmeg</u> seed (<u>Myristica fragrans Houttuyn</u>). In this case we had to polish the seed to put it in a 12 mm tube, since an average <u>nutmeg</u> kernel sized about a 3 cm (in length) x 2 cm (in diameter). A very complex but well resolved ^{13}C nmr spectrum was observed even for this harden seed (Figure 3).

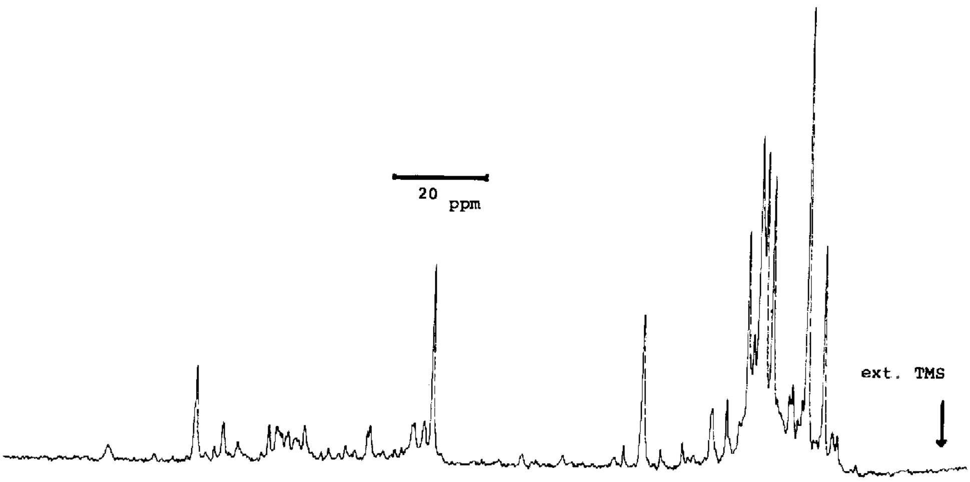

Figure 3. ^{13}C Nmr spectrum of whole <u>nutmeg</u>, obtained from Spice Island Inc., South San Francisco, California, which was polished to fit in a nmr tube.

We have not analyzed the spectrum in detail yet, but there should not be any essential difficulties to assign each peaks to the essential oils and triglycerides contained in <u>nutmeg</u>, and to study the histological and motional states of these compounds in the tissues.

Finally we stress that this kind of histological information can not be obtained by a conventional analytical method which requires separation procedures unavoidably. Further accounts of our studies will be published shortly.

The authors wish to thank Drs. Katsumi Ajisaka and Nobuya Nagashima for their discussions.

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