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^{13}C NMR STUDIES OF THE INTACT PLANT TISSUES. CYTOPLASMIC AUCUBIN AND SUCROSE IN A SINGLE SEED OF AUCUBA JAPONICA

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Aucuba japonica Thunberg, a Japanese name *aoki*, is one of the most common garden plants in Japan, and it bears lots of small fruits which turn beautiful red during a winter-time. The plant is noted also for aucubin, an iridoid glucoside contained in the whole plant. As the first account for the gross nmr approach to phytochemistry, we wish to report here the ^{13}C nmr spectroscopic study of a intact *aoki* fruit.

As a prolate-shaped *aoki* fruit usually has a size of about 2 X 1.5 cm when it ripes and is slightly too large to be accommodated in a 12 mm sample tube. Therefore a cleaned seed sized about 1.5 X 1 cm was subjected for the nmr measurements. A ripe seed, harvested in April, which looked like a semi-transparent amber bead, gave a surprisingly well-resolved ^{13}C nmr spectrum after a rather short time of signal accumulation (Figure 1).

The spectrum consists of 12 lines arising from sucrose, $C_{12}H_{22}O_{11}$, and of 15 lines due to aucubin, $C_{15}H_{22}O_9$, in the cytoplasm. We, in fact, could observe 26 lines all together, which means only two resonances were overlapped to each other. All of the 27 lines, however, were clearly observed and assigned to respective carbons through a series of single frequency proton decoupling experiments, in which an incremental 10 Hz offset was applied to the decoupling output over a range of 400 Hz (4 ppm). By this a little elaborating experiment on a single *aoki* seed, we successfully determined most of the proton chemical shifts of aucubin and sucrose in cytoplasm. We should point out an

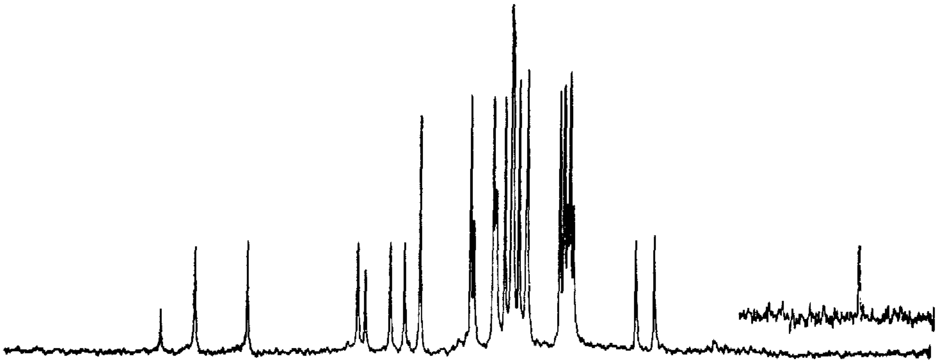
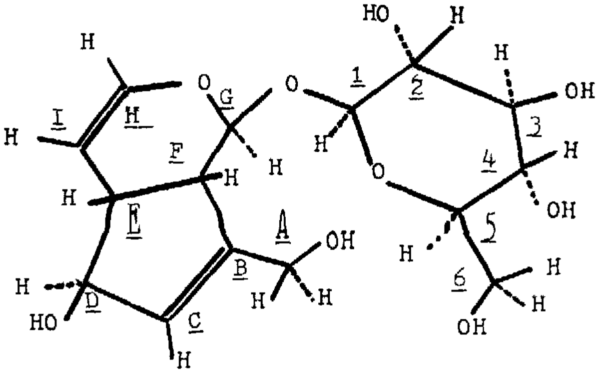


Figure 1. The 25.2 MHz ^{13}C nmr spectrum of a single aoki seed. Spectrum width, 5,000 Hz; 7,500 transients were accumulated. (insert) TMS in carbontetrachloride, measured right after the spectrum of seed was recorded.



The structure of aucubin (1,2).

attempted direct ^{1}H nmr measurement for a single aoki seed did not show any sharp lines except a broad hump at a slightly higher field to the water peak.

These data, together with the ^{1}H and ^{13}C chemical shifts obtained for the aqueous solutions of the "free" aucubin and sucrose, allow us an unprecendented oppotunity to compare the molecular structures of plant metabolites in cytoplasm to those of the isolated compounds.

In Table I, listed are the ^{13}C and ^{1}H nmr chemical shifts of aucubin in an intact seed and those of the free aucubin dissolved in water. Both the ^{13}C and ^{1}H shifts are nearly identical in the two states. This observation strongly implies that the structure as well as conformation of aucubin are identical in both states. A little difference, about 0.3 ppm found between the ^{13}C shifts of the cytoplasmic and free aucubin is due most likely to a

Table I. The ^{13}C and ^{1}H chemical shifts of aucubin in an intact aoki seed and in water. ^{13}C shifts refer to the resonance of TMS in CCl_4 contained in the other 12 mm tube. ^{1}H shifts refer to an internal TMP (sodium 2,2,3,3,-tetradeutero-3-trimethylsilyl-propionate).

	^{13}C nmr, ppm		$^{13}C-\{^{1}H\}$, ppm		^{1}H nmr
	seed	free	seed	free	free
A	61.0	61.3	c	4.23	4.30
B	148.3	148.5	---	---	---
C	130.0	130.3	5.85	5.85	5.85
D	82.1	82.3	4.60	4.54	4.54
E	43.8	44.1	2.70	2.66	2.77
F	47.7	48.0	3.00	2.95	3.13
G	99.8	100.1	4.75	4.76	4.78
H	141.1	141.3	6.30[b]	6.30[b]	6.30
I	106.7	106.9	5.15	5.07	5.12
1	96.8	97.1	5.25	5.19	5.25
2	74.2	74.6	c	3.28	d
3	77.6[a]	77.9[a]	c	3.31[a]	d
4	71.1	71.4	c	3.35	d
5	77.3[a]	77.5[a]	c	3.49[a]	d
6	62.2	62.5	c	3.70	d

[a] Assignment of these resonances may be reversed. [b] Shifts refer to the H *proton which was assumed to be 6.30 ppm from* TMP. [c] Accurate shifts were not obtained due to the overlapped sucrose peaks. [d] Too complex to be analyzed.

bulk susceptibility difference between two states. A similar chemical shift difference thus was observed for sucrose too. We however could not detect any additional ^{13}C nmr shift differences between the cytoplasmic and free sucrose.

Therefore the structures of aucubin and sucrose in the intact aoki seed are considered to be identical to those in aqueous solutions of these compounds. Theoretically, however, there might be a possibility that the structures or conformations of plant metabolites in cytoplasms might be different from those of the isolated compounds.

We have measured the ^{13}C nmr spectrum of the left-overs, namely skin and flesh, of ripe aoki fruits as well after the seeds were pitted out. A little surprisingly only glucose and fructose resonances were observed, and neither sucrose nor aucubin were detected by nmr. This is in marked contrast with the fact that there are no glucose or fructose in the seed. With a green immature aoki fruit, however, we could not find out any resonances but those due to aucubin in the seed. These observation may indicate that glucose and fructose, which have been formed and stored in the flesh as the fruits ripe up, turn into sucrose while they transport themselves into the seeds. It seems to be quite plausible to assume that sucrose synthetase is localized in the thin seed case which separates a seed from flesh. At any rate the observations are closely related and therefore very useful to the physiology of this plant.

The other interesting gross chemical application of ^{13}C nmr may be such as following. As we were able to observe aucubin and sucrose to start with, the isolation of these compounds from fruits were conveniently followed by monitoring the ^{13}C nmr in each single steps of the procedures in order not to leak or spoil the compounds. We therefore were able to get five grams of crude aucubin out of a 100 grams of fresh ripe aoki fruits. Obviously this kind of monitoring will be useful to know a proper harvesting time or even to improve plants genetically.

We have observed a variety of compounds, including sugars, polysaccharides, fats, essential oils, glycosides or so on by ^{13}C nmr of the various intact plant tissues. We could also observe phytic acid in plant seeds by ^{31}P nmr spectroscopy. These results and scope of the gross chemical application of high resolution nmr will be given shortly in separate papers.

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