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BIOSYNTHETIC STUDIES WITH CARBON-13: EFFECTIVE USE OF A PARAMAGNETIC ION IN THE FT-^{13}CNMR SPECTRA OF HELICOBASIDIN

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From incorporation studies with ^{14}C-acetates,[1,2] 2-^{14}C-mevalonic acid[2] and 2-^{14}C-4R-^{3}H-mevalonic acid,[3,4] several pathways for the biosynthesis of the fungal sesquiterpene helicobasidin (1) have been proposed. These routes commence with farnesyl pyrophosphate (2) which cyclizes to an intermediate at the cuprenene (3) oxidation level possessing a cyclohexenyl ring. In one route this ring is directly converted to the dihydroxyquinone ring of helicobasidin. In another, it is oxidized to an aromatic ring giving cuparene (4). The aromatic ring then would serve as the precursor for the quinone ring of the metabolite.

Since evidence was lacking on the precise location of the C-14 labeled carbons in the quinone ring, we used 90% enriched dl-2-^{13}C-mevalonic acid lactone and FT-^{13}CNMR to locate the ^{13}C-label in helicobasidin.

In helicobasidin biosynthesized from a C-2 labeled mevalonic acid precursor, randomization of the label between C-8 and C-10 in the quinone ring should occur since proton tautomeric shifts in helicobasidin (1a and 1b) make the C-8 and C-10 positions equivalent. No distinction can therefore be made between the intermediacy of cuprenene or cuparene in the biosynthetic process.

Addition of 2-^{13}C-mevalonic acid to growing cultures of Helicobasidium mompa, precultured on a sawdust medium, afforded, after three weeks, helicobasidin, which was converted with acetic anhydride in pyridine to the more chloroform soluble diacetate to facilitate cmr measurements.

As expected, the FT-^{13}CNMR spectrum of natural abundance helicobasidin diacetate exhibited considerable variation in signal intensities (Fig. 1). Such signal intensity differences, which are caused by differences in the nuclear Overhauser enhancement (NOE) and T_1 of individual carbons are well established.[5,6]

The spectrum of helicobasidin diacetate derived from 2-^{13}C-mevalonate showed enhanced upfield signal intensities at 27.9 (C-12) and 39.2 ppm (C-4), but no definitive enhancement of intensity was observable for the weaker downfield C-8 and C-10 signals. C-8 and C-10 signals were greatly diminished relative to those of the hydrogen bearing carbons. The addition of the paramagnetic tris(acetylacetonato)chromium(III)[$Cr(acac)_3$] (0.1M) to the labeled sample facilitated the assignment of enriched sites. A FT-^{13}CNMR spectrum was now obtained, which had enhanced signals of equal intensity for C-8 and C-10 (Fig. 2).[7,8] This result is in agreement with the expected equal distribution of labels at C-8 and C-10.

$Cr(acac)_3$ quenches the variable NOE and decreases the T_1's to more uniform values and thereby eliminates the two major causes of intensity differences in FT-^{13}CNMR spectra. Although the addition of the $Cr(acac)_3$ did quench the NOE for all carbons, the S/N of the proton bearing carbons was not significantly decreased and that of the non-proton bearing carbons was actually increased about three-fold. This is the result of the much shorter spin-lattice relaxation times which allowed the use of more efficient spin flip angles.

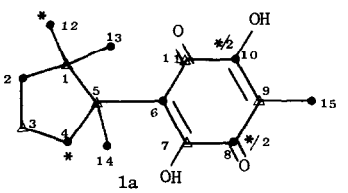
Two additional ^{13}C-labeled samples of helicobasidin diacetate were biosynthetically prepared from 1-^{13}C-acetate and 2-^{13}C-acetate. From the known pathway of helicobasidin biosynthesis from acetate via mevalonate and farnesyl pyrophosphate (2), the carbon shift positions were determined (Table). These samples on addition of 0.1 M $Cr(acac)_3$ had FT-^{13}CNMR spectra with more uniform intensities and a pronounced increase in the S/N of non-proton bearing carbons which facilitated identification of the enriched sites (Fig. 2).

The use of paramagnetic ions in FT-^{13}CNMR biosynthetic studies thus promises to help overcome the inability to detect low ^{13}C-enrichments, a serious limitation of the cmr method. Further applications of this technique will be forthcoming.

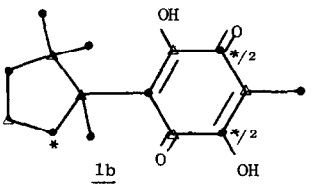
Table ^{13}C-Shift Assignments of Helicobasidin Diacetate

Assignments	δc (ppm)[a]	Assignments	δc (ppm)[a]
15	8.9 (q)	5[c]	52.5 (s)[d]
$OCO\underline{C}H_3$	20.3 (q)	9	129.4 (s)
3	20.6 (t)	6	141.8 (s)
$OCO\underline{C}H_3$	20.7 (q)	7	149.7 (s)
13[b]	23.6 (q)	10	150.3 (s)
14[b]	25.5 (q)	$O\underline{C}OCH_3$	167.7 (s)
12	27.9 (q)	$O\underline{C}OCH_3$	168.4 (s)
4	39.2 (t)	8	180.2 (s)
2	41.6 (t)	11	181.3 (s)
1[c]	45.7 (s)[d]		

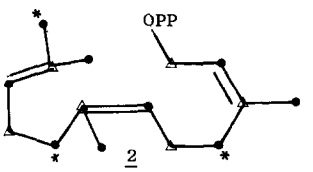
a Measured in $CDCl_3$, downfield from TMS. q, t and s indicate multiplicities from off resonance measurement.
b Assignments may be reversed.
c Assignments may be reversed.
d $J_{^{13}C-^{13}C}$ = 33.4 Hz.



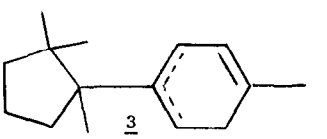
1a



1b



2



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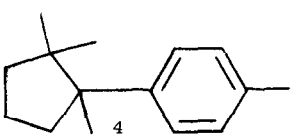

4

- from C-2 of acetate
△ from C-1 of acetate
* from C-2 of mevalonate

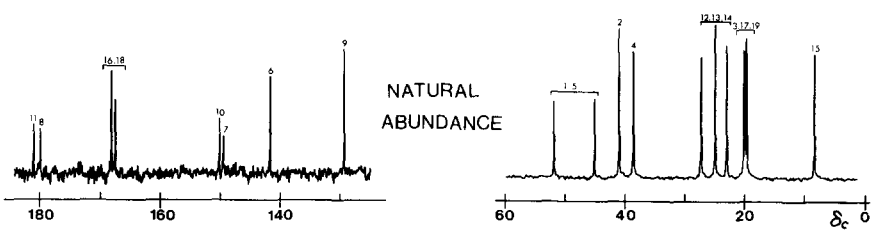

FIGURE 1

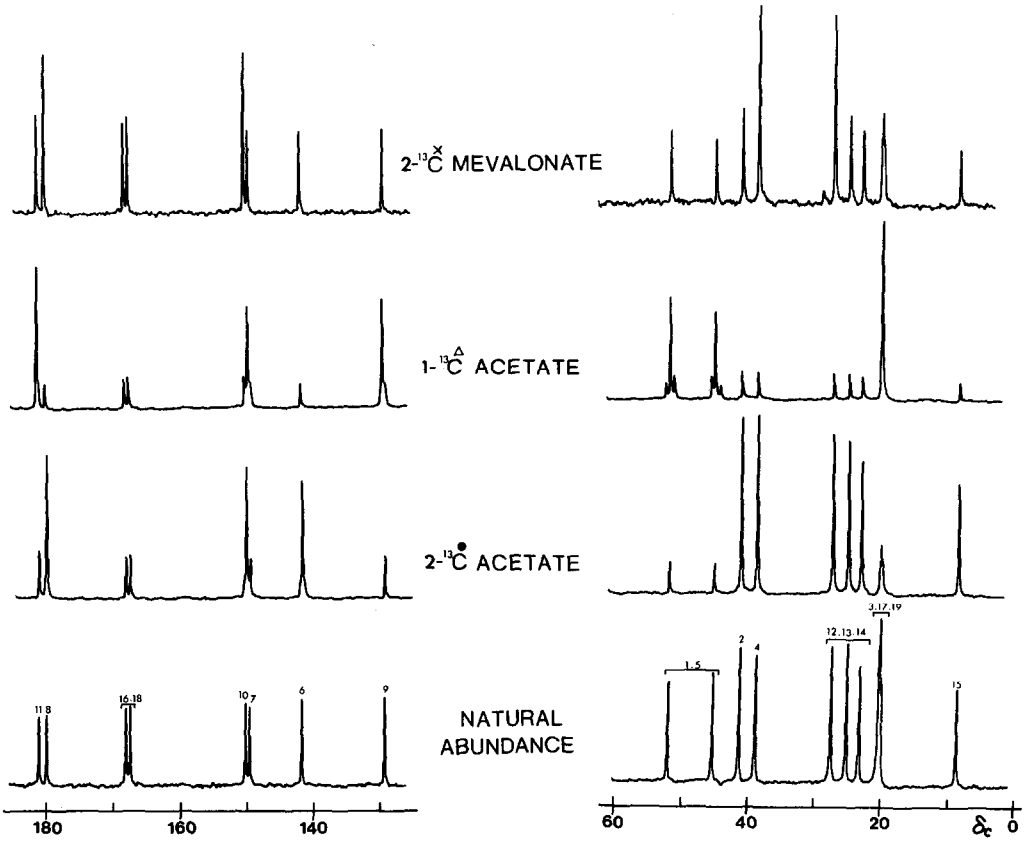

FIGURE 2

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