Tetrahedron Letters No. 25, pp 2097 - 2100, 1976. Pergamon Press. Printed in Great Britain.

BIOSYNTHESIS OF FOMANNOSIN FROM 1,2-^{13}C-ACETATE

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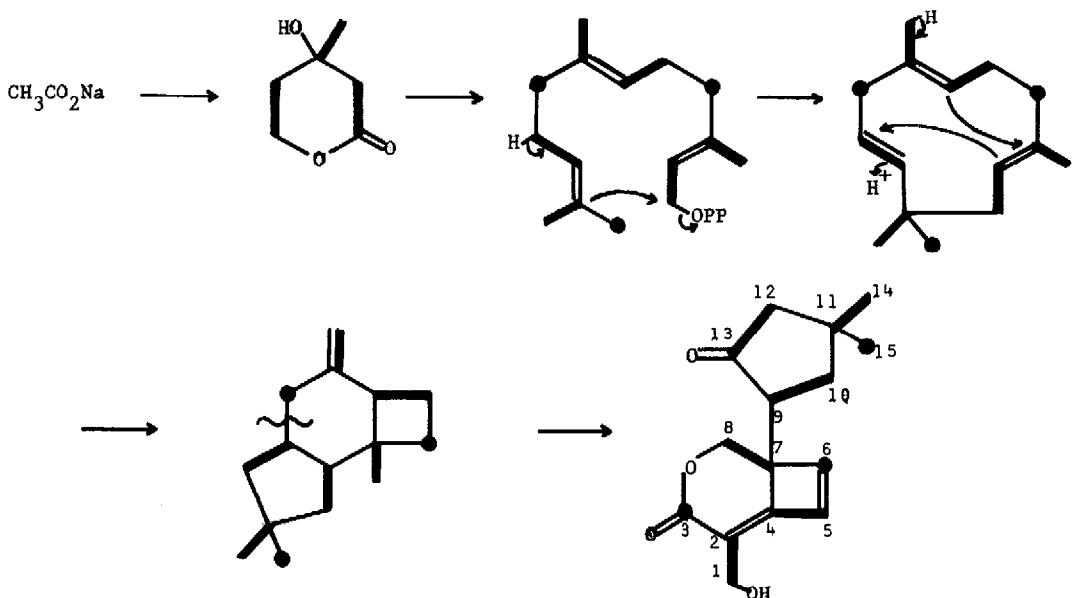
(Received in USA 14 April 1976; received in UK for publication 10 May 1976)

Fomannosin (1) has been implicated in the phytopathogenic activity of the economically important wood rot fungus Fomes annosus which affects pine stands in the Southeastern United States. The structure of this unusual sesquiterpene was established by spectroscopic methods and by X-ray crystallographic analysis of the p-bromobenzoylurethane derivative of dihydrofomannosin.[1] Recently a synthetic approach to fomannosin has been described.[2] Biogenetic speculations[3] have centered on a humulene precursor which might cyclize to a tricyclic compound related to illudol followed by oxidative cleavage of the appropriate bond (Scheme 1). Although biosynthetic studies have been reported for the biogene-



Scheme 1

1

tically related illudins,[4] coriolins,[5] and hirsutic acid,[6] to date there have been no reports of biosynthetic studies of 1 itself. In connection with our own interest in the application of stable isotopes to isoprenoid biosynthetic studies,[7] we have been examining the biosynthesis of 1 and our results are reported below.

The proton-noise decoupled carbon magnetic resonance spectrum of fomannosin exhibits 15 signals which are readily assigned with the aid of off-resonance decoupling (SFORD) and by comparison with known chemical shift data and shift parameters.[8] The assignments are listed in Table I.

A total of 62 mg of sodium 1,2-^{13}C-acetate (90% enriched), diluted with 186 mg of unlabeled acetate and mixed with 1.76 x 10^6 dpm of sodium-^{14}C acetate was administered under sterile conditions to two 42 day old surface cultures of F. annosus, FSLD 63, (two 800 ml Roux bottles, 250 ml broth per bottle).[9] After an additional 8 days the cultures were harvested, and the culture filtrate extracted with chloroform. The fomannosin (121 mg) was isolated by column chromatography on silica gel and repurified by preparative layer chromatography.

The CMR spectrum of enriched 1 exhibited six pairs of spin-coupled doublets appearing as satellites about the (enhanced) natural abundance singlets, as well as three enhanced singlets. The observed ^{13}C enrichment at each carbon was ca. 0.5%, consistent with that calculated from measurement of the ^{14}C content of 1, assuming incorporation of nine acetates per mole of fomannosin. The results are presented in Table I and illustrated schematically in Scheme 1.

As has been demonstrated previously,[10] when 1,2-^{13}C-acetate is used as a biosynthetic precursor of isoprenoid metabolites, all carbons derived from C-3 and C-3' and C-4 and C-5 of mevalonate give rise to coupled doublets in the cmr spectrum of ^{13}C-labeled metabolites, provided that the paired atoms remain connected. Adjacent carbon atoms derived from distinct molecules of acetate will not show spin-spin coupling as long as there is a significant pool of unlabeled acetate, either administered externally or endogeneously generated.[11] Furthermore since C-1 of mevalonate is lost as carbon dioxide in the formation

of isopentenyl pyrophosphate, all carbons in any subsequent metabolite which are derived from C-2 of mevalonate give rise to enhanced singlets. The cmr spectrum of labeled 1 indicates that carbons 3 and 6 as well as the low field methyl group of fomannosin are derived from C-2 of mevalonate. All other paired carbon atoms of acetate remain intact resulting in the observed set of coupled signals. Our results therefore are consistent with previous biogenetic speculations on the origins of fomannosin. Work is in progress to examine further mechanistic and stereochemical details of this biosynthetic pathway.[12]

Table 1. ^{13}C NMR of Fomannosin[a]

Carbon	δ_c[b]	J_{cc}(Hz)[c]	Carbon	δ_c	J_{cc}(Hz)
1	58.4t[d]	52	9	46.5d	35
2	114.2s	51	10	38.3t	36
3	166.1s	s	11	33.7s	35
4	155.1s	44	14	28.1q	35
5	140.2d	44	12	53.4t	38
6	146.5d	s	13	219.3s	37
7	52.7s	37	15	29.7q	s
8	73.7t	38			

[a]Bruker WP-60, 15.08 MHz; spectral width 3906 Hz, acquisition time 1.048 sec, pulse delay 2.0 sec, pulse width 3.5 μs, 26104 transients, 0.92 M solution in $CDCl_3$, 10 mm sample tube. [b]TMS = 0.00 ppm. [c]Observed coupling of satellite doublets for ^{13}C-enriched 1. [d]Multiplicity in SFORD spectrum: s = singlet, d = doublet, t = triplet, q = quartet.

References and Notes

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9. Culture conditions for production of fomannosin by F. annosus are described in Bassett et. al., ref. 1. FSLD 63 was a gift of Dr. George Kuhlmann of the Forestry Sciences Laboratory, Durham, North Carolina.

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11. Failure to dilute doubly labeled precursors with sufficient unlabeled material may often lead to complications in interpretation of spectroscopic data as a result of excessive multiple labeling of the products. See, for example, R. C. Paulick, M. L. Casey, D. F. Hillenbrand, and H. W. Whitlock, J. Am. Chem. Soc., 97, 5303 (1975).

12. We would like to thank Mr. Louis Messerle for carrying out early experiments with F. annosus. This work was supported financially by a grant from the National Institutes of Health (1 RO1 GM22172-01).