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BIOSYNTHETIC STUDIES WITH CARBON-13: INCORPORATION PATTERN OF 1,2-^{13}C-ACETATE INTO THE FUNGAL SESQUITERPENE OVALICIN

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We report the use of doubly labelled acetate and FT-^{13}C-NMR in the determination of the biosynthetic origin of ovalicin (1)(1) (graphinone), a sesquiterpene found in cultures of *Pseudeurotium ovalis*.

The fungus was cultured in media fortified with 90% enriched 1,2-^{13}C-acetate that was diluted two-fold with unlabelled acetate. The FT-^{13}C-NMR spectrum (Figure 1) of the labelled metabolite showed twelve characteristic(2) carbon satellite signals. These satellite signals which are caused by ^{13}C-^{13}C coupling occur on both sides of the center natural abundance peaks and only appear when doubly labelled acetic acid is incorporated. Since twelve such carbon signals were observed in the spectrum of labelled ovalicin, six molecules of doubly labelled acetic acid had been incorporated. The position of incorporation of these six acetate units, shown in Scheme 1, was determined from known carbon chemical shifts,(3) carbon-carbon coupling constants(3) and the carbon signal multiplicities of the off-resonance decoupled spectrum of the unlabelled metabolite. The chemical shift and coupling data of the labelled ovalicin are presented in Table I.

The signals for the C-2, C-8 and C-12 carbons of 1 have enhanced intensities relative to the natural abundance C-16 methoxyl signal and appear as singlet signals without carbon-carbon coupling (see Figure 1). This result confirms the acetate-mevalonate pathway in ovalicin biosynthesis since cleavage of the C-1 to C-2 bond in mevalonic acid occurs during its conversion to isopentyl pyrophosphate and the C-2, C-8 and C-12 carbons of 1 are derived from C-2 of mevalonic acid.

The pattern of acetate incorporation is consistent with the acetate to mevalonate, to cis-farnesyl pyrophosphate route. The biosynthetic route may proceed by cyclization of the farnesol derivative to α-bergamotene (2) followed by cyclobutane ring opening to ovalicin as shown in Scheme 1.(1d,4)

Our result is in agreement with the proposed biosynthetic conversion of mevalonic acid to the related antibiotic fumagillin 3(5). Incorporation of 2-^{14}C-mevalonic acid had been established by ozonolysis of fumagillol (4) and location of the theoretical one third of the radioactivity in the ozonolysis product, acetone.(5)

These results show the utility of the 1,2-^{13}C acetate method in the study of terpenoid biosynthesis since with a single feeding experiment the biosynthetic origin of all fifteen carbons of 1 was established.

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Table I CARBON CHEMICAL SHIFTS (δc)[a] OF OVALICIN

Carbon Number and Multiplicity[b]	^{13}C-^{13}C Coupling Constants (Hz)
C_1 (s) (60.8) - C_{13} (t) (51.4)	32
C_2 (t) (30.4)	
C_3 (t) (36.8) - C_4 (s) (207.1)	38
C_5 (d) (86.4) - C_6 (s) (78.9)	38
C_7 (s) (60.5) - C_{14} (q) (14.4)	46
C_8 (d) (59.4)	
C_9 (t) (27.2) - C_{10} (d) (118.5)	43
C_{11} (s) (135.8) - C_{15} (q) (18.0)	42
C_{12} (q) (25.8)	
C_{16} (q) (57.0)	

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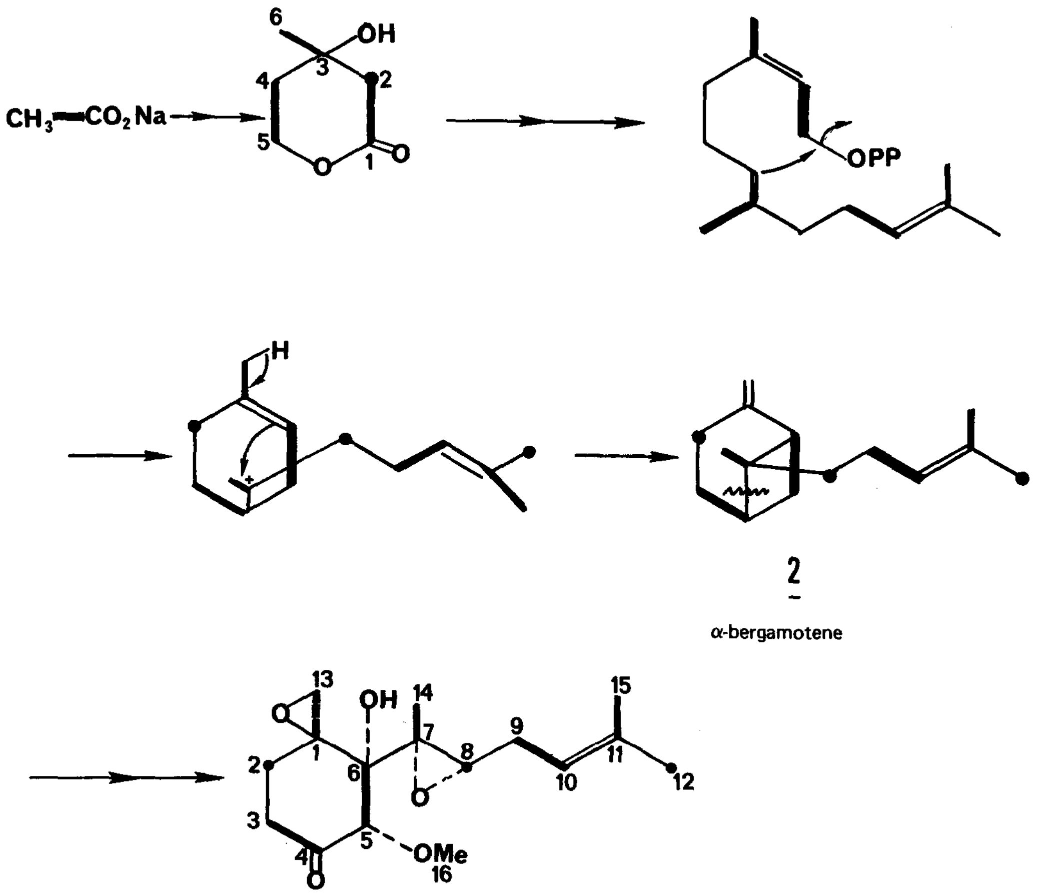
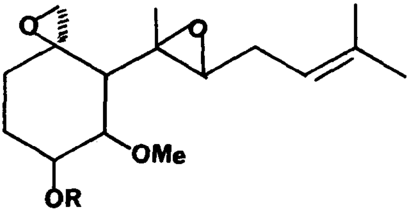
a. Determined in deuterochloroform in ppm downfield from internal TMS.

b. (s) - singlet; (d) - doublet; (t) - triplet; (q) - quartet. Obtained from off-resonance decoupled spectrum of unlabelled ovalicin.



1

SCHEME I



3 R = CO(CH=CH)$_4$CO$_2$H
4 R = H

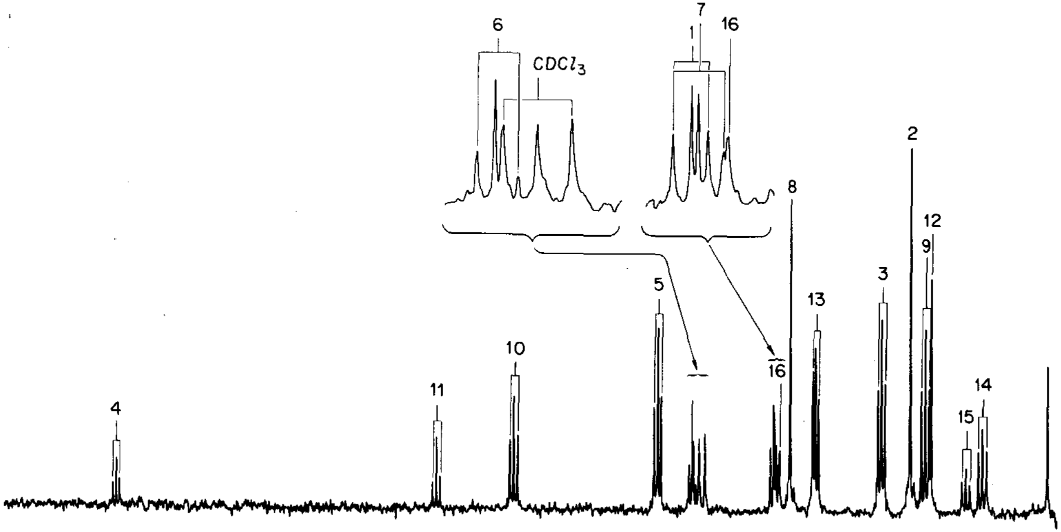


Figure 1 Proton noise decoupled FT-^{13}C-NMR spectrum of ^{13}C-labelled ovalicin (*80 mg in 0.7 ml* CDCl$_3$) at 25.2 *MHz, spectral width, 6000 Hz,* 9933 acquisitions, 16K data points.