METABOLITES OF PYRENOMYCETES. XV[1]. BIOGENESIS OF THE ANTITUMOR ANTIBIOTIC, (+) R-AVELLANEOL

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We recently reported (1) the structures of a broad spectrum antibiotic, avellaneol (1), a metabolite of *Hypocrea avellanea* (Rogerson & Carey). Subsequent tests conducted at the National Cancer Institute showed it to be mildly active towards PS388 lymphocytic leukemia in mice.

The six- and three-carbon units present in avellaneol, though common in aromatic compounds derived from acetate and shikimic acid pathways, are very rare in aliphatic compounds. A mixed pathway (2) involving condensation of a four-carbon tricarboxylic acid cycle (TCA) intermediate, with a true polyketide chain and subsequent elimination of the carboxyl function has been proposed for several metabolites having similar skeleton. These conclusions were based on radioactive ^{14}C acetate incorporation followed by degradation experiments. Simpson and Holker (3) used a vastly superior method of incorporating ^{13}C acetates and ^{13}C-nmr for detection. Based on these studies they proposed a different mechanism in which a true polyketide chain first undergoes a chain-fission; subsequent rearrangement leads to the formation of compound **2**, 3-epoxypropyl-5-hydroxy-6-methyl-5, 6-dihydro-2H-pyranone. Long-range carbon-carbon coupling constants (ca. 7Hz) in the ^{13}C-nmr clearly demonstrated that one acetate unit had split and assumed a 1:3 relationship, thus giving credence to the proposed biogenetic pathway.

We studied the incorporation of $^{13}C_1$ and $^{13}C_2$ acetates in avellaneol. In close similarity to the pyrone **2**, the unusual labelling pattern involving enrichment of C_2, C_4, C_6 and C_8 when $^{13}C_1$ was incorporated and of C_1, C_3, C_5, C_7 and C^9 when $^{13}C_2$ acetate was incorporated was observed. This involves a tail-to-tail linkage which is rare. Sodium $^{13}C_2$ propionate failed to incorporate. We did not get any satisfactory results in our $^{13}C_1$-$^{13}C_2$ doubly labelled acetate incorporation studies.[2] However, our present results with singly labelled acetates taken together with the negative results from propionate incorporation suggest that avellaneol was formed biogenetically in a similar pathway (scheme 1) as pyrone **2**.

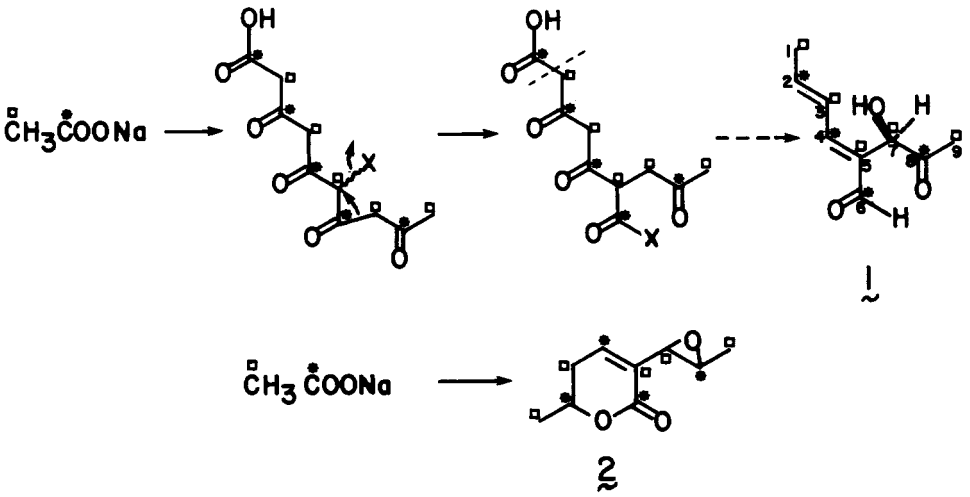
EXPERIMENTAL

CULTURE.—A four-week-old culture of *H. avellanea* was inoculated into 18 Fernbach flasks, each containing glass wool and 400 ml of a glucose-yeast medium. After 10 days of incubation in the dark, 1g 90% ^{13}C enriched $^{13}C_1$ sodium acetate was introduced into one third of the flasks. The same amounts of $^{13}C_2$ sodium acetate and $^{13}C_2$ sodium propionate were introduced into 6 each of the remaining flasks. Incubation was then continued in the dark at 26° for another 12 days, after which culture liquid and mycelia were separately harvested. Specimens of *H. avellanea* are on deposit at the Herbarium of the New York Botanical Garden.

ISOLATION OF AVELLANEOL.—The culture liquid (2.4 liters) was extracted three times with equal volumes of ethyl acetate and the dried extract was chromatographed on one hundred times its weight of silicagel with chloroform-methanol (10:1) used for elution. Avellaneol is very unstable in air; therefore,

[1]For part XIV see: M. S. R. Nair, S. T. Carey and J. C. James, *Tetrahedron*, 37, 2445 (1981).

[2]Our preliminary work involving $^{13}C_1$-$^{13}C_2$ sodium acetate incorporation resulted in very low yields (less than 2 mg/liter) of avellaneol and inadequate ^{13}C enrichment to detect second order C-C coupling. Further work could not be done as all the authors left the laboratory where fermentation was being conducted.



Scheme 1.

low-bar column chromatography under 15 psi of ultra high purity nitrogen was employed for its isolation. About 25 mg of avellaneol, an oil, was obtained per liter of culture liquid.

^{13}C NMR SPECTRA.—Proton-noise and CW off-resonance decoupled ^{13}C-nmr spectra reported in an earlier paper (1) had established the chemical shifts for each of the carbons. Examination of the spectra after the incorporation of ^{13}C acetates showed that the carboxyl carbon was incorporated on C_2, C_4, C_6 and C_8 (δ 126.7, 145.9, 193.1 and 206.6 ppm) and methyl of the acetate on carbons C_1, C_3, C_5, C_7 and C_9 (δ 19.3, 153.3, 135.1, 72.0 and 24.9 ppm). In the acetate incorporation experiments, the ^{13}C enrichment of avellaneol was ca. 2%. In the case when $^{13}C_3$ propionate was added to the culture medium, the relative intensities of the ^{13}C-nmr signals were the same as in unlabelled avellaneol indicating that no incorporation occurred.

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3. T. J. Simpson and J. S. E. Holker, *Tetrahedron Lett.*, 4693 (1975); T. J. Simpson, *ibid.*, 175 (1975).