Biosynthetic Studies with Carbon-13: Fourier Transform Nuclear Magnetic Resonance Spectra of the Metabolite Avenaciolide

By MASATO TANABE,* TAKASHI HAMASAKI, and YOSHIYUKI SUZUKI
(*Stanford Research Institute, Menlo Park, California* 94025)
and LEROY F. JOHNSON
(*Analytical Instrument Division, Varian Associates, Palo Alto, California* 94303)

Summary ^{13}C N.m.r. spectra of ^{13}C-labelled avenaciolides show its biosynthetic origin from 3-oxododecanoic acid and succinic acid.

SEVERAL schemes for the biosynthetic origin of the bislactone avenaciolide (I), a metabolite of *Aspergillus avenaceus*,[1] have been suggested.[2] We report from ^{13}C-labelling studies that avenaciolide is biosynthesized as shown in the Scheme.

^{13}C-Enriched avenaciolides were prepared in separate experiments from growing cultures of *Aspergillus avenaceus*, G. Smith (CMI, 16140), in Czapek Dox medium fortified with sodium [1-^{13}C]acetate (90%) and sodium [2-^{13}C]-acetate (60%). The F.t. ^{13}C n.m.r. spectra of the ^{13}C-enriched bislactones isolated were obtained in chloroform solution. From the material labelled by sodium [2-^{13}C]-acetate, the enhanced signals observed for the alternating carbons (C-1, -3, -5, and -7) of the n-octyl side chain in the upfield region of the spectrum indicated enriched sites. Their chemical shift assignments are in accord with the reported shift values for octan-2-ol.[3] The two oxygenated carbons C-9 and C-13 are enriched sites and in the expected shift region. The two unsaturated carbons C-11 and C-15 are also labelled, and carbon–carbon coupling is observed with $J(^{13}C–^{13}C)$ 75 Hz, which is in agreement with a reported coupling constant of 67·6 Hz for ethylene. In the absence of off-resonance decoupling data, the lower field 134·9 p.p.m. signal, with a reduced intensity relative to the C-15 signal

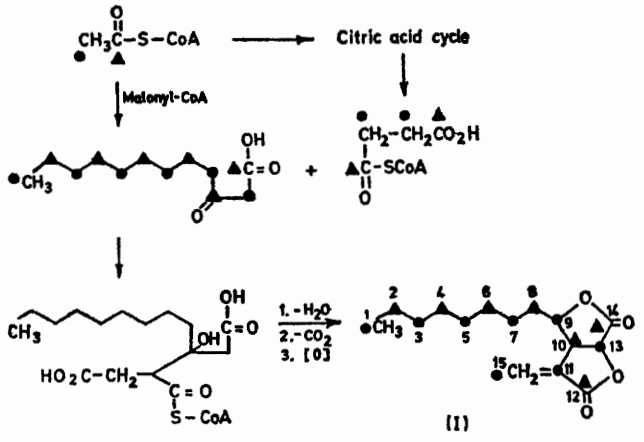
at 126·2 p.p.m., is assigned to the quaternary C-11 which has no directly bonded protons. The succinic acid origin of C-15, C-11, and C-12 is nicely confirmed by the carbon–carbon coupling observed for C-11, C-15, which would be expected if [2-^{13}C]acetate was incorporated into this acid in the citric acid cycle.

TABLE. ^{13}C *N.m.r. data for avenaciolide*

Carbon No.	δ_C (p.p.m.)[a] [2-^{13}C]acetate	[1-^{13}C]acetate
1	14·0	
2		22·6
7	24·9	
4		29·1
5	29·1	
6		29·1
3	31·8	
8		35·8
10		44·0
9	74·6[b]	
13	85·6[b]	
15[d]	126·2[c]	
11[d]	134·9[c]	
12[d]		169·9
14		170·3

[a] Downfield from Me_4Si. [b] These values can be reversed. [c] Carbon–carbon coupling J 75 Hz. [d] These positions are labelled by both [1-^{13}C]- and [2-^{13}C]-acetate since succinic acid cycles through the citric acid cycle with dispersion of the label.

In the avenaciolide from sodium [1-^{13}C]acetate the alternate carbons (C-2, -4, -6, -8, -10, and -14) are labelled, since they show enhanced signal intensities. The shift data are summarized in the Table. These labelling studies indicate that 3-oxododecanoic acid (Scheme), formed by the acetate–malonate pathway, is an intermediate in the biosynthesis of avenaciolide. The keto-acid condenses with succinylCoA which is generated from the citric acid cycle to give a condensation product that is then transformed to avenaciolide. The intensity of the C-14 carbonyl signal is higher than that of the C-12 carbonyl peak,



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confirming the biosynthetic origin of C-12 from [1-^{13}C]-acetate *via* succinic acid that has been diluted in the citric acid cycle. The higher level of enrichment observed for the 3-oxododecanoic acid-derived moiety in avenaciolide indicates acetate is converted more rapidly into this intermediate than into succinic acid.

Support of this work by the US Public Health Service is acknowledged.

(*Received*, 1*st January* 1973; *Com.* 009.)

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