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THE BIOSYNTHESIS OF PENTALENOLACTONE

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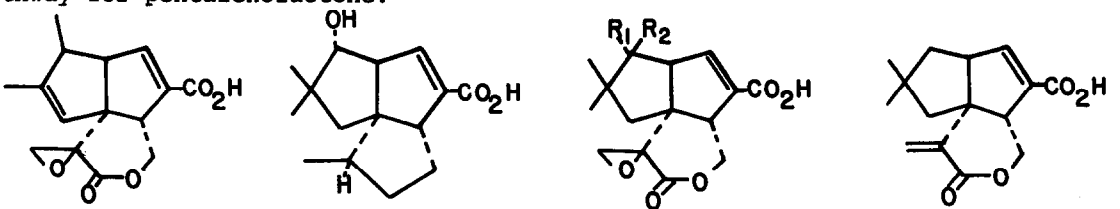
ABSTRACT: Feeding of [UL-$^{13}C_6$]-glucose, an in vivo precursor of [1,2-$^{13}C_2$]-acetyl-CoA, gave pentalenolactone in which the pattern of ^{13}C-enrichments and couplings supported a mevalonoid biosynthetic pathway.

The isolation and structure determination of the Streptomyces antibiotic pentalenolactone (1) has stimulated both chemical and biosynthetic investigations of this structurally novel metabolite.[2] Seto's group has identified the methyl esters of a group of metabolites, pentalenic acid (2) and the epoxy lactones pentalenolactone H (3) and G (4), which are potential intermediates or shunt metabolites of the biosynthetic pathway.[3] Our own group has recently reported the isolation of yet another related substance, pentalenolactone E (5).[4] At the same time Shirahama and Matsumoto and their collaborators, as part of an extensive study of biogenetically modeled cyclizations of humulene (6) and derived protoilludyl cations, have reported the synthesis of the tricyclic sesquiterpene hydrocarbon 7.[5] We report below a biosynthetic study, using [UL-$^{13}C_6$]-glucose as an in vivo precursor of [1,2-$^{13}C_2$]-acetyl-CoA, which supports a mevalonoid biosynthetic pathway for pentalenolactone.



Initial attempts to incorporate several of the usual isoprenoid precursors, including [2-^{14}C]-acetate, [2-^{14}C]-mevalonate, and [2-^{14}C, 5-$^{3}H_2$]-mevalonate, under a variety of feeding regimens and with two different strains of Streptomyces, were uniformly unsuccessful, giving rise to pentalenolactone which was devoid of radioactivity.[6] While failure of bacterial cultures to take up exogeneous mevalonate is not uncommon,[7] we were apparently faced with overcoming permeability or compartmentation difficulties. In order to overcome these obstacles, we therefore turned to the use of uniformly ^{13}C-labeled-[UL-$^{13}C_6$]-glucose. Such a precursor would have a number of attractive features, including the ability to penetrate the

cell wall and act as an *in vivo* precursor of [1,2-$^{13}C_2$]-acetyl-CoA, an intermediate which has been a particularly powerful tool for the study of isoprenoid and polyketide biosynthetic pathways.[8] Although [UL-$^{13}C_6$]-glucose has recently been used in an investigation of thiamine biosynthesis in which the distribution of label was examined by mass spectrometry,[9] the present study is the first use of this substrate in connection with multiple-label ^{13}C nmr.

In order to minimize extraneous couplings due to excess intramolecular labeling,[10] the precursor [UL-$^{13}C_6$]-glucose (1.35 g, 85 atom % ^{13}C) was mixed with unlabeled glucose (1.35 g), along with [6-^{14}C]-glucose (1.74 x 10^8 dpm) as internal standard and fed to 2.4 L of *Streptomyces* UC5319 which had been grown in a modified medium for 30 hr at 28°C. After an additional 42 hr the resulting pentalenolactone was isolated and rigorously purified as the methyl ester. A total of 18.5 mg of 1-Me was obtained. The measured specific activity (2.78 x 10^6 dpm/mmol) coresponded to a 0.1% incorporation of glucose and a calculated ^{13}C enrichment of 2.2% per labeled site.

Complete ^{13}C nmr assignments for pentalenolactone methyl ester have been reported by Takeuchi.[11] We have independently confirmed these assignments by single-frequency off-resonance decoupling and correlation with the ^1H nmr spectrum by the method of Birdsall.[12] These assignments are further corroborated by the observed ^{13}C-^{13}C couplings listed in Table 1. The ^{13}C nmr spectrum of the biosynthetically labeled pentalenolactone methyl ester strongly suggests a mevalonoid biosynthetic pathway, illustrated in the figure. As summarized in Table 1, carbons 7 and 11 each give rise to enhanced singlets, as predicted for carbons derived from C-2 of mevalonate which loses its paired acetate atom from C-1 of mevalonate in the formation of isopentenyl pyrophosphate.[8] The signals for C-6 and 13, C-5 and 12, C-9 and 10, and C-3 and 4 of pentalenolactone appear as enhanced and coupled doublets. The paired doublets corresponding to C-2 and C-15 suggest that the allylic methyl of 1 is derived from C-3' of mevalonate. The spectrum does exhibit one unanticipated and rather curious feature. Carbon-1, which appears as a triplet, J = 36 Hz, and a doublet, J = 36 Hz, each centered on the enhanced natural abundance singlet, is coupled not only to C-8, as expected, but also to C-14, which has presumably migrated from C-2 at some stage. The triplet corresponds to those species in which C-14, C-1, and C-8 are all labeled while the doublet arises from molecules in which only the C-1, C-8 or C-1, C-14 pairs are labeled. The 14-methyl carbon must be derived from the same glucose molecule as the pair of carbons to which it has become attached, since no other extraneous couplings are evident in the remainder of the spectrum. While the addition of unlabeled glucose to the precursor can prevent two acetates from different glucose precursors from being labeled in the same molecule of product, there is no way *a priori* to prevent two acetates from the *same* glucose from finding each other if there is little or no dilution by an endogeneous pool of triose phosphates at the site of glycolysis and mevalonate synthesis.[13] Whereas the implication that such primary metabolic activities are

more compartmentalized than previously suspected remains speculative, the alternative explanation for the extra coupling is even less compelling. Formation of pentalenolactone from a fifteen-carbon acyclic pyrophosphate in which the end of the chain is derived from intact two- and three-carbon intermediates coming from glucose would be entirely unprecedented and neither account for the formation of geminal methyl metabolites 2-5 nor explain the presence of the 2,3-double bond in pentalenolactone itself.

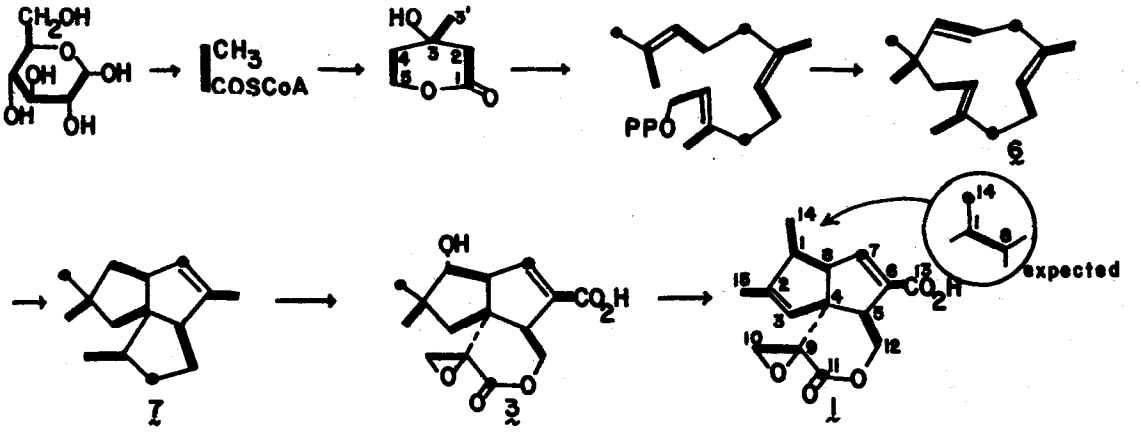


Table 1. ^{13}C NMR of Pentalenolactone Methyl Ester[a]

Carbon	δ_c[b]	J_{cc}, Hz[c]	Carbon	δ_c	J_{cc}, Hz
1	44.5 d[d]	36 d, 36 t[e]	10	47.1 t	33
8	56.7 d	36	11	169.4 s	s
14	15.5 q	35	12	67.7 t	34
2	147.9 s	44	5	51.0 d	37
15	14.6 q	46	6	133.4 s	75
3	122.3 d	43	13	164.3 s	76
4	59.2 s	44	7	146.1 d	s
9	59.1 s	32	16	51.8 q	s

a) Bruker WP-60, 15.08 MHz; spectral width 4000 Hz, 4K data points, quadrature detection, 40° pulse, 4.0 s pulse delay, 44288 transients, 18.5 mg in 0.5 ml $CDCl_3$. The observed signal enhancements, calculated by comparing the normalized total area of each set of signals with that of the natural abundance methyl of the methyl ester, ranged from 1.5-2.5% (average enrichment 1.9 ± 0.3%) in reasonable agreement with the value calculated from the measured ^{14}C activity, assuming two acetates per glucose. b) TMS = 0.00 ppm. c) Observed coupling (±1 Hz) for satellite doublets for ^{13}C-enriched 1-Me. d) Multiplicity in SFORD spectrum: s = singlet, d = doublet, t = triplet, q = quartet. e) Area t/d ≈ 2.

Pentalenolactone appears to be biogenetically related to the important class of humulene-derived sesquiterpenes which includes fomannosin,[14] illudins,[15] marasmic acid,[16] hirsutic acid,[17] and the coriolins.[18] Further experiments to test the intermediacy of compounds 2-5 and to settle the origin of the extra couplings are in progress.[19]

References and Notes

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6. Strains of S. roseogriseus, ATCC 12414 failed to produce pentalenolactone using a variety of media. Satisfactory production was achieved using Streptomyces UC 5319 obtained from the Upjohn Co.
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