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BIOSYNTHESIS OF SECALONIC ACID A. THE PATHWAY INDICATED BY A ^{13}C NUCLEAR MAGNETIC RESONANCE STUDY OF ACETATE INCORPORATION

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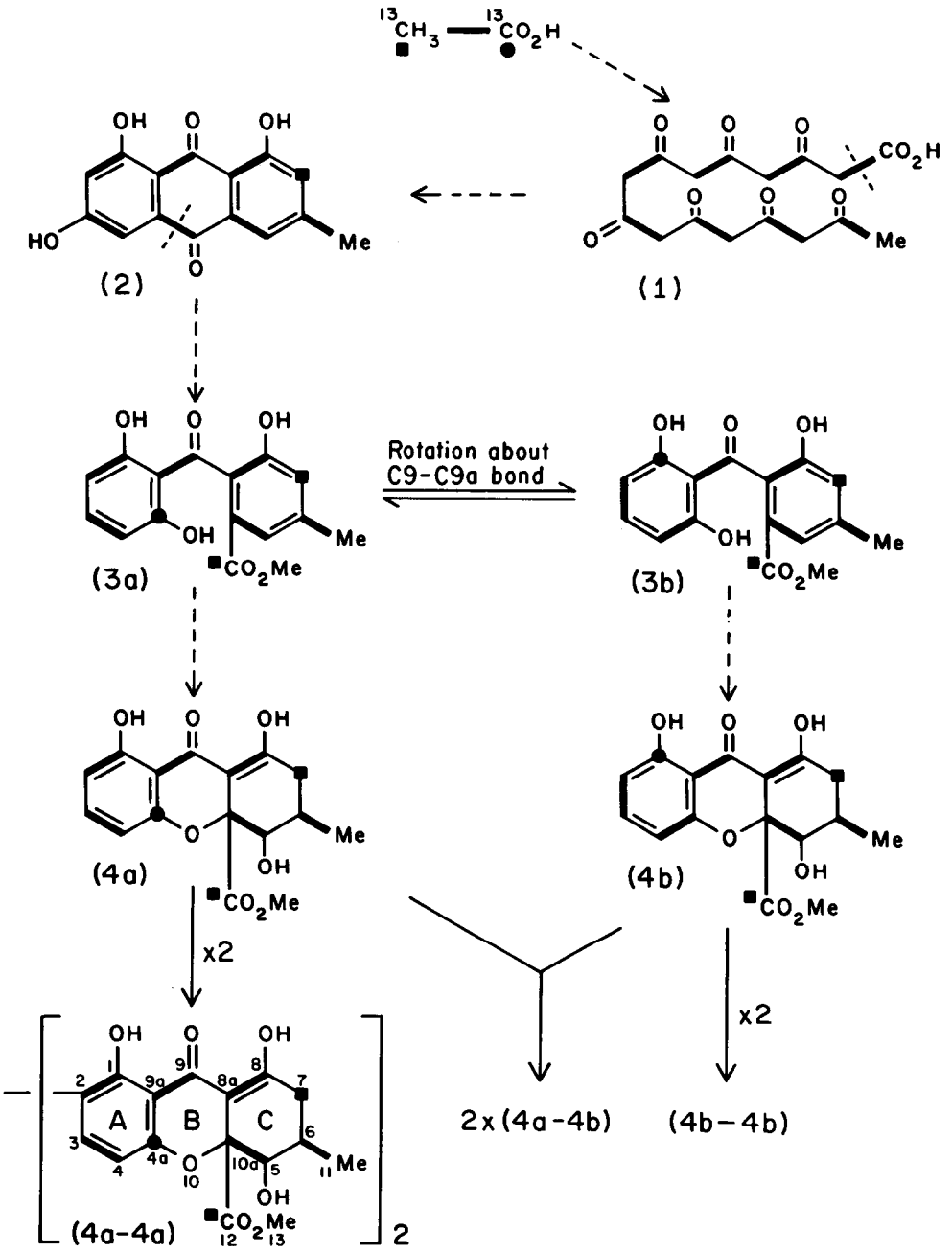
Studies on ergochrome biosynthesis with ^{14}C-labelled acetate[1,2] and ^{14}C- and ^{3}H-labelled emodin (2)[2,3] are consistent with the pathway octaketide (1) → anthraquinone (2) → benzophenone carboxylic ester (3) → tetrahydroxanthone (4) → ergochrome (e.g. 4a-4a), but do not exclude dimerization of 2 or some related tricarbocyclic intermediate before the xanthone ring is formed. If 3 is an intermediate, either hydroxyl group in the symmetrically substituted ring can participate in the cyclization. Such alternative ring closures can be detected if the intermediate has been labelled by (1,2-^{13}C)acetate, because they generate three species of enriched secalonic acid A differing in the arrangement of intact ^{13}C-^{13}C units in their structures (Scheme). Birch and colleagues have recently reported that the use of doubly-labelled acetate indicates similar alternatives in the biosynthesis of ravenelin[4]. If xanthone-ring formation followed dimerization the labels could not redistribute, and only one isotopically-labelled species of secalonic acid A (4-4) could exist. We have used ^{13}C nmr analysis of secalonic acid A biosynthesized from (1,2-^{13}C)acetate to distinguish between these alternatives.

Administration of sodium (1,2-^{13}C)acetate (90% ^{13}C-enriched) to Pyrenochaeta terrestris gave secalonic acid A which showed characteristic satellite resonances in the ^{1}H-broadband decoupled ^{13}C nmr spectrum, indicating the presence of directly bonded pairs of ^{13}C-enriched carbon atoms (Table 1). Only sixteen signals were observed in the natural-abundance ^{13}C nmr spectrum because secalonic acid A is a symmetric molecule. Resonances were assigned on the basis of chemical shift correlations, evidence from high-resolution and single ^{1}H-frequency off-resonance decoupled spectra, isotope chemical shifts observed on replacing the hydroxyl

[1]NRCC No. 16476.


13CH3—13CO2H
(1)
(2)
(3a)
(3b)
Rotation about C9-C9a bond
(4a)
(4b)
x2
x2
2x(4a-4b)
(4b-4b)
(4a-4a)
2
A
B
C

hydrogens with deuterium, and the ^{13}C-^{13}C spin-spin coupling information obtained from secalonic acid A enriched with (1,2-^{13}C)acetate.

Eight bonded ^{13}C-^{13}C pairs were identified by matching coupling constants, and enrichments (see Table 1) were calculated as reported previously[5]. Only C-7 and C-12, which were both labelled by [2-^{13}C]acetate in another experiment, and C-13 lacked satellite resonances, and the labelling pattern was consistent with the biosynthetic pathway outlined in the Scheme. It is noteworthy that C-4 was ^{13}C-^{13}C coupled to C-3 and C-4a, and C-2 to C-1 and C-3, with each of the four ^{13}C-^{13}C units possessing about half the enrichment of similar units in rings B and C. Thus the five carbons were enriched from the incorporation of two intact acetate units, the enrichment distribution arising from rotation about the two-fold axis of symmetry in the trisubstituted ring of intermediate 3. This rotation, which interchanges carbons on opposite sides of the axis, can occur only if, at some stage, 3 is not bound rigidly to an enzyme surface. While the heterocyclic rings in ergochromes are labile under appropriate conditions[6], the observed distribution of ^{13}C label in secalonic acid A precludes dimerization at any stage before the benzophenone intermediate is formed and reaches rotational equilibrium. In secalonic acids the equilibration is detected only by introducing cryptic asymmetry into the benzophenone through ^{13}C labelling. No 2,4'- or 4,4'-isomers are present in cultures of P. terrestris, excluding the possibility that the xanthone rings might open and reform after the two half-molecules of secalonic acid have been linked.

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Table 1

^{13}C nmr data for secalonic acid A labeled by [1,2-^{13}C]acetate

Carbons	δ_C(TMS)[a] (ppm)	$^1J_{CH}$[b,c] (Hz)	$^1J_{CC}$[c] (Hz)	Average enrichment (%)
C-1,1'	159.97		} 69.3	0.09±0.01
C-2,2'	118.14			
C-3,3'	140.80	159.3	} 58.6	0.09±0.04
C-4,4'	107.81	165.0	} 57.2	0.09±0.03
C-4a,4a'	159.88		} 72.3	0.08±0.03
C-5,5'	76.60	143.2		
C-10a,10a'	86.19		} 39.7	0.17±0.05
C-6,6'	30.54	131.2		
C-11,11'	18.43	125.6	} 36.0	0.18±0.02
C-8,8'	178.55			
C-8a,8a'	102.59		} 70.9	0.16±0.01
C-9,9'	187.84			
C-9a,9a'	107.46		} 55.2	0.18±0.02
C-7,7'	36.71	127.5		
C-12,12'	171.10			
C-13,13'	52.75	147.7		

a. Spectrometer, Varian XL-100/15 Fourier transform; frequency 25.16 MHz; spectral width 5120 Hz; acquisition time 1.6 s; time-constant for weighting free-induction decay 0.8 s; flip angle 34°; pulse width 15 μs; 201 mg in 0.5 ml pyridine-d_5; internal reference $(CH_3)_4Si$; tube diameter 5 mm; temp 30°C; internal lock to lowest-field ^2H resonance of solvent; ^1H-decoupling field $\gamma H_2/2\pi$ ca. 3800 Hz, phase modulated from 0 to 180° at 150 Hz for broadband decoupling.

b. Measured from high-resolution spectrum, ^1H-decoupling field applied for 1.6 s between data acquisition periods.

c. Error ± 0.6 Hz.