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BIOSYNTHESIS OF KINAMYCIN D. INCORPORATION OF [1,2-^{13}C]ACETATE AND OF [2-$^{2}H_3$,1-^{13}C]ACETATE

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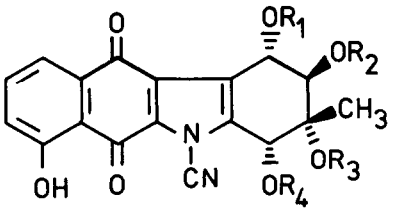
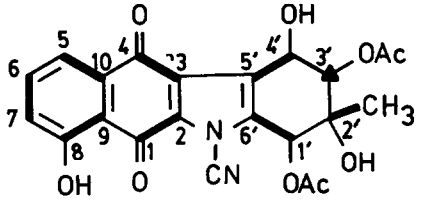
Abstract: Results from the incorporation of [1,2-$^{13}C_2$]- and [2-$^{2}H_3$,1-^{13}C]acetates into kinamycin D. indicate the involvement of only unsymmetrical intermediates; the identity of some of these are suggested.

The origin of the antibiotics kinamycins A-D, 1-4, metabolites of *Streptomyces murayamaensis* sp. nov. Hata *et* Ohtani,[2] presented an unusual biosynthetic problem. Retrobiosynthetic analysis indicated a naphthoquinone and a "C_7N" unit as reasonable biosynthetic intermediates; each of these could come from two fundamentally different metabolisms. Naphthoquinones can be derived from acetate via a polyketide intermediate[3] or from shikimic acid and α-ketoglutarate,[4] while the necessary C_7N unit could come from a shikimate-type pathway[5] or, possibly, from acetate via decarboxylation of a substituted o-toluic acid. Ōmura has presented evidence for the incorporation of acetate into 4.[6] We now report results that define the origin of the kinamycin D skeleton.

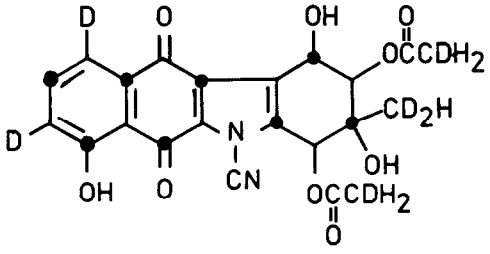
S. murayamaensis was grown by inoculating two 200 mL production broths[7] in 1-liter Erlenmeyer flasks with 10 mL of a 48 hour seed broth.[8] These were incubated at 25-26°C on a rotary shaker (1" stroke) at 300 rpm. Fifteen hours later a mixture of sodium [1,2-$^{13}C_2$]acetate[9] (197 mg) and sodium (1-^{14}C)acetate[10] (11.7 μCi) was divided into equal portions and added sterilely to the fermentations. After an additional thirty-three hours' incubation, the mycelia were removed by filtration through cheese cloth. The filtrate and the mycelial cake were extracted separately with benzene until the extracts were colorless. The combined extracts were dried over Na_2SO_4, concentrated to dryness,



	R_1	R_2	R_3	R_4
1	H	Ac	Ac	Ac
2	H	H	Ac	H
3	Ac	Ac	H	Ac
4	H	Ac	H	Ac



4a



4b

and the amount of total kinamycins determined by UV spectroscopy. Purification by preparative tlc ($CHCl_3$:EtOAc=3:2) yielded a mixture of kinamycins C and D, which was further purified to afford pure 4a (3.2 mg). The 100 MHz ^{13}C NMR spectrum ($CDCl_3$)[11] yielded the labeling pattern shown in structure 4a and summarized in Table 1.[12,13] All carbon resonances were enriched (average 3.1% enrichment per position),[14] and all except C-3' showed coupling to one other carbon. C-3' was 2.5% enriched over natural abundance.

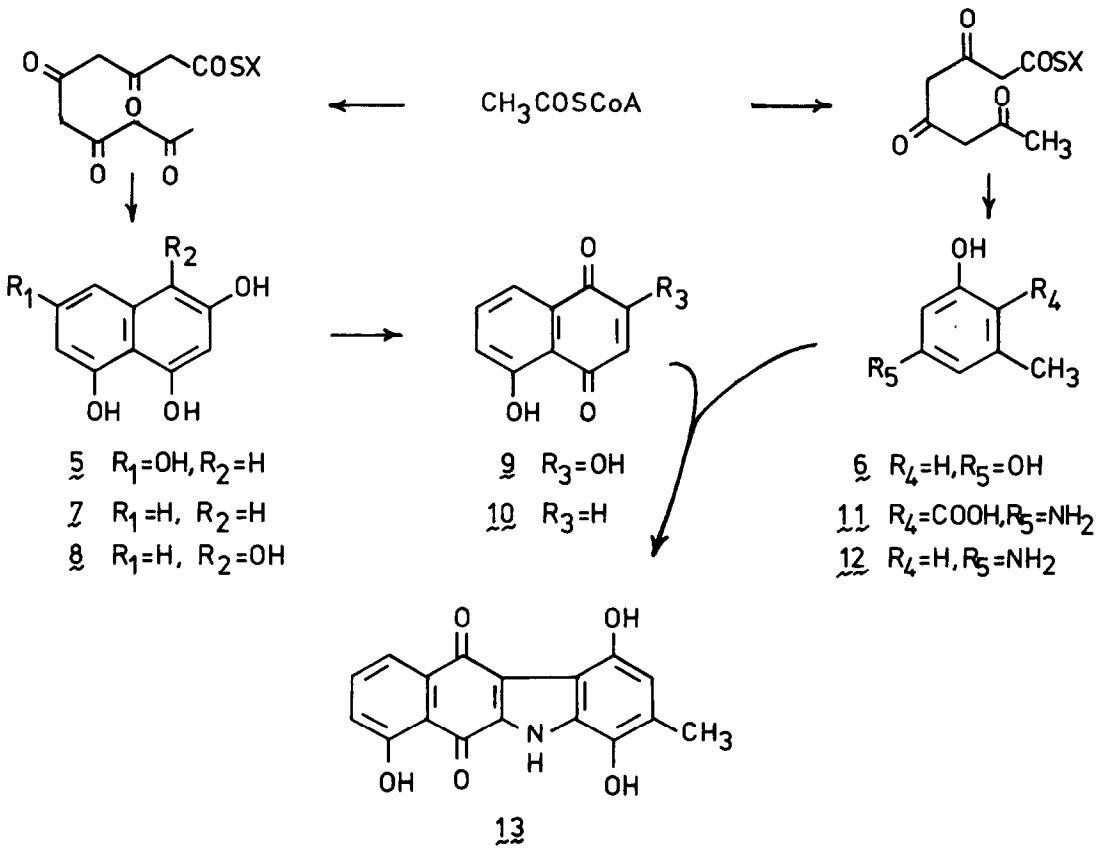
Sodium [2-$^2H_3$,1-^{13}C]acetate (492 mg) mixed with sodium (1-^{14}C)acetate (10.9 μCi) was next fed to five flasks, each containing 200 mL of fermentation, again thirteen hours after inoculation. After extraction with benzene, the kinamycin mixture was chromatographed on silicic acid. Elution with $CHCl_3$:EtOAc=5:1 and recrystallization from ethyl acetate afforded 20 mg of pure 4b that was analyzed by ^{13}C NMR. Deuterium induced β-isotope shifts[15] were observed for the resonances of C-6 (0.10 ppm), C-10 (0.07 ppm), C-2' (0.05 and 0.10 ppm), and the acetate carbonyls (0.03 ppm each), revealing the retention of deuterium at H-5, H-7, the C-2' methyl, and the acetate methyls respectively. No isotope shift for C-6', the partner to C-1', was detectable.

These results establish the derivation of the kinamycin skeletons entirely from acetate by way of non-symmetrical intermediates, as shown in Scheme 1. Had naphthalene 5 been involved, two sets of coupled resonances would have been observed for kinamycin; similarly, orcinol 6 can be ruled out as a D-ring precursor. Thus, 2,4,5-trihydroxynaphthalene, 7, is expected to be the first free A/B ring intermediate, followed by 1,2,4,5-tetrahydroxynaphthalene, 8, and either 2-hydroxyjuglone, 9, or juglone, 10.[4] 3-Amino-5-hydroxytoluic acid, 11, and 3-amino-5-hydroxytoluene, 12, are likely candidates as D-ring precursors. Finally, the lack of deuterium at C-1' indicates that the D-ring

Table 1. Chemical Shifts and Jcc Coupling Constants of Kinamycin D Derived from [1,2-$^{13}C_2$]Acetate.

C Position	Chemical Shifts (ppm)	Jcc (Hz)	C Position	Chemical Shifts (ppm)	Jcc (Hz)
1	183.6	63.0	5	120.3	62.0
4	180.8	60.5	9	115.6	63.7
8	162.4	63.7	3'	75.7	Singlet
6	136.2	58.5	2'	73.7	41.6
10	133.8	62.0	1'	71.3	51.2
2	132.8	63.0	4'	67.3	47.4
5'	132.1	47.4	$-CH_3$	18.2	41.6
3	129.0	60.5			
6'	127.8	51.2			
7	124.3	58.5			

SCHEME 1



5 R_1=OH, R_2=H
7 R_1=H, R_2=H
8 R_1=H, R_2=OH

9 R_3=OH
10 R_3=H

6 R_4=H, R_5=OH
11 R_4=COOH, R_5=NH_2
12 R_4=H, R_5=NH_2

13

is still aromatic after introduction of oxygen at this position as indicated in hypothetical intermediate 13. The testing of these hypotheses as well as determining the origin of the kinamycin oxygens is currently study.

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6. K. Ajisaka, H. Takeshima, and S. Ōmura, *J.C.S. Chem. Commun.* 1976, 571, and private communication from Professor Ōmura.
7. The recipe provided by Ōmura was modified, and now consists of 2% glucose, 0.5% peptone, 0.1% yeast ext., 0.5% beef ext., 0.5% NaCl, and 0.3% $CaCO_3$.
8. The recipe provided by Ōmura was modified, and now consists of 1.0% glycerol, 1.0% soybean meal and 0.3% NaCl.
9. Purchased from Merck Sharp and Dohme Canada, Limited.
10. Purchased from ICN Pharmaceuticals, Inc.
11. Obtained on a Bruker Am 400 NMR spectrometer using a 5mm multinuclear probe tuned for ^{13}C and set for Waltz decoupling of protons.
12. The assignments described in the previous paper were fully consistent with the feeding results except those for C-5' and C-6' which must now be reversed.
13. The labeling pattern of kinamycin C was the same as that of kinamycin D.
14. In none of our spectra have we seen evidence of a resonance for the cyano carbon.
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