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THE ANSAMYCIN ANTIBIOTIC ACTAMYCIN. II. DETERMINATION OF THE STRUCTURE USING CARBON-13 BIOSYNTHETIC LABELLING

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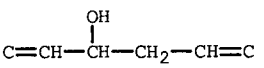
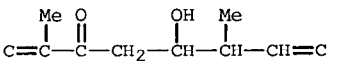
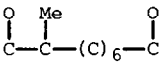
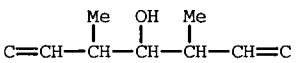
Summary: *The structure of the ansamycin antibiotic actamycin is defined by analysis of biosynthetically ^{13}C-enriched and natural abundance n.m.r. spectra, in conjunction with previous degradative evidence.*

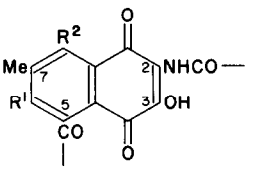
The preceding paper[1] describes the use of deuterium labelling in conjunction with degradative and spectroscopic studies to establish the structural segments (1), (2), (3), (4) and (5a) or (5b) in the ansamycin antibiotic[2] actamycin. We now present evidence from a biosynthetic ^{13}C-labelling experiment and n.m.r. spectra which defines the structure (15) of actamycin.[3]

(1) 

(2) 

(3) 

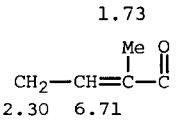
(4) 

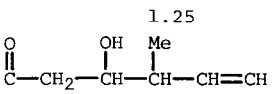


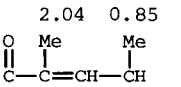
(5a) $R^1 = OH$, $R^2 = H$

(5b) $R^1 = H$, $R^2 = OH$

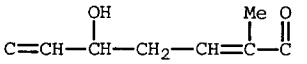
The presence in actamycin of the segments (6), (7) and (8) follows from proton chemical shift and spin decoupling studies (270 MHz, $CDCl_3$).

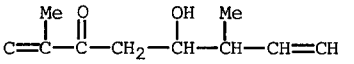
(6) 

(7) 

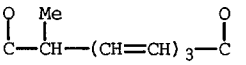
(8) 

Since both ^{1}H and ^{13}C n.m.r. spectra show only two methylene groups, one allylic and one adjacent to carbonyl, then segments (1) and (6) can be merged to form (9), and (2) and (7) can be superimposed to form (10).

(9) 

(10) 

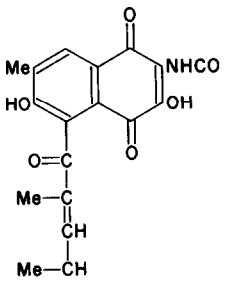
Furthermore, the segment (3), which was defined by oxidation following catalytic reduction,[1] must carry a triene and no CH_2 group. It must therefore originate from the structure (11) in actamycin.

(11) 

The ^{13}C n.m.r. spectrum of actamycin shows only two ketonic resonances, at δ 203.7 and 202.1 ppm (67.89 MHz, $CDCl_3$). One ketone has the environment represented by segment (10). The second carbonyl is linked to the naphthoquinonoid nucleus as in (5a) or (5b), and must be the carbonyl group which terminates either segment (8) or (9). A simple biosynthetic labelling experiment determines the environment of this ketone and also distinguishes the two possible nucleus structures (5a) and (5b).

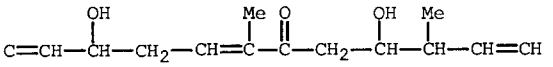
Polyketide chain biosynthesis in ansamycins is initiated by 3-amino-5-hydroxybenzoic acid, which provides the nitrogen-carrying ring.[4] Sequential addition of activated propionate and acetate units to this initiator, followed by cyclisation, yields the 5-carbonyl-7-methyl-substituted bicyclic nucleus {e.g., (5a) or (5b)} typical of naphthalenoid ansamycins.[2] [^{13}C]-Actamycin was produced by fermentation of Streptomyces sp. E/784 in the presence of sodium [1-^{13}C]propionate. The ^{13}C n.m.r. spectrum of this material showed six resonances enriched to approximately twice natural abundance, while the remaining resonances were unaltered. The enriched signals included the ketonic carbonyl at δ 203.7, a secondary hydroxyl carbon at δ 75.5, and notably three olefinic methine carbons at δ 145.8, 143.1 and 133.3 ppm. Single frequency proton-carbon decoupling established that the olefinic proton at δ 5.97 in the structural segment (8) was attached to an enriched olefinic carbon, whilst the corresponding carbon in the enone of segment (9), carrying the proton at δ 6.71, was not enriched. Furthermore, the phenolic hydroxyl carbon, δ 160.9 ppm, was also enriched.

These labelling results locate the phenolic hydroxyl group at C-6 of the naphthoquinone, as in (5a), and establish that the aryl carbonyl group terminates not segment (9) but segment (8), as in partial structure (12).

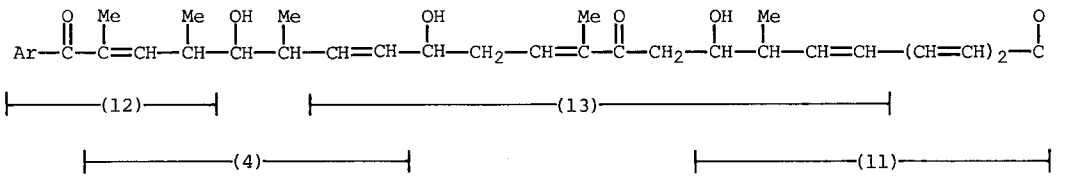


(12)

Since there are only two ketone functions, segment (9) must therefore be merged with segment (10) to give partial structure (13).

(13) 

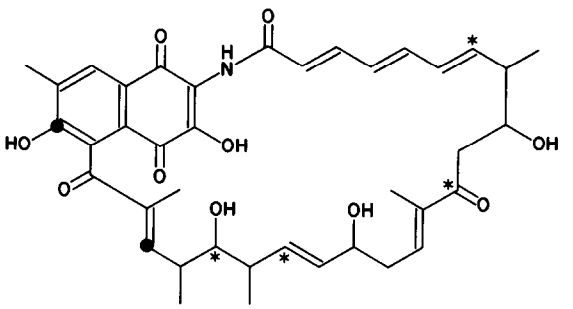
An ansa bridge known from mass[1] and ^{13}C n.m.r. spectra to contain 23 in-chain carbon atoms must now be formed from segments (12), (4), (13) and (11), which in total contain 32 in-chain atoms. This overlap of 9 in-chain atoms can be achieved in only one way (14).



(14)

As required for formation of the amide linkage back to the nucleus, the chain (14) terminates in an oxygenated carbon from segment (11).

The structure of actamycin without regard to stereochemistry can now be written as (15). The two carbons specifically proven to be labelled by [1-^{13}C]propionate are dotted. The four other carbons expected on biogenetic grounds to be labelled are asterisked, and match precisely the remaining four enriched carbon resonances described above in the ^{13}C n.m.r. spectrum of [1-^{13}C]propionate-labelled actamycin.



(15)

Actamycin (15) is closely related to naphthomycin,[5] in which the quinonoid hydroxyl of actamycin is replaced by chlorine and the ansa bridge carries an additional C-methyl group α to the amide carbonyl. The present work emphasises the potential of heavy isotopes in structure determination.

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