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THE ANSAMYCIN ANTIBIOTIC ACTAMYCIN. I. DEFINITION OF STRUCTURAL FEATURES BY DEUTERIUM LABELLING

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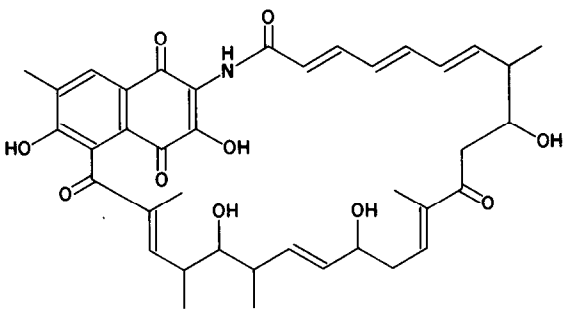
Summary: *The ansamycin antibiotic actamycin is shown to contain the nucleus (2a) or (2b) and the ansa bridge segments (7), (8), (9) and (10) by spectroscopic and degradative studies in conjunction with deuterium labelling.*

We present in this and the following paper evidence based upon the use of spectroscopy and heavy isotopes which leads to the structure (1) for actamycin,[1] a new ansamycin antibiotic[2] m.p. 190-192° obtained from an unidentified *Streptomyces* sp. (Lepetit strain E/784).

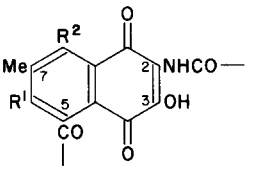
(1)



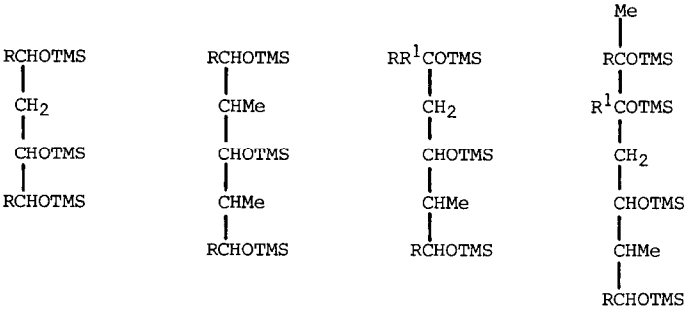
The molecular formula of actamycin was established as $C_{39}H_{45}NO_{10}$ by analysis and high resolution mass spectrometry of the parent compound and its dimethyl ether, $C_{41}H_{49}NO_{10}$. Redox properties together with carbonyl ^{13}C resonances at δ 182.0 and 179.4 ppm (67.89 MHz, $CDCl_3$) indicated a quinonoid nucleus, necessarily a naphthoquinone in view of n.m.r. spectral data and electronic absorption (EtOH-HCl) at 284 sh, 303, 346 sh and 443 sh nm (log ε 4.39, 4.43, 4.09 and 3.08, respectively). The presence of a ^{13}C resonance at δ 168.0 typical of an amide carbonyl, in conjunction with highly unsaturated N-containing ions of composition $C_{12}H_8NO_5$ (m/z 246) and $C_{14}H_{12}NO_5$ (m/z 274) in mass spectra of actamycin and its dimethyl ether, suggested a dihydroxy-naphthoquinonoid ansamycin structure. Naphthalenoid ansamycins are characterised by an aliphatic ansa bridge linked by amide and carbonyl (or latent carbonyl) groups to the 2- and 5-positions, respectively, of the bicyclic nucleus.[2] They all carry a 7-methyl substituent, observable in the ^1H n.m.r. of actamycin as a resonance at δ 2.41 weakly coupled to an adjacent aromatic proton at δ 7.90. This evidence establishes the structure (2a) or (2b) for the nucleus of actamycin. In confirmation of this 3-hydroxy-

1,4-naphthoquinone structure, actamycin is a relatively strong acid soluble in aqueous $NaHCO_3$, and one of the methoxyl groups in its dimethyl ether is hydrolysed by aqueous Na_2CO_3.

(2a) R^1 = OH, R^2 = H
(2b) R^1 = H, R^2 = OH



^1H and ^{13}C n.m.r. data show that the C_{28} ansa bridge contains six olefinic bonds. In view of this extensive unsaturation, structural segments of the bridge were defined by ozonolysis. The per-ozonide was reduced directly with lithium aluminium hydride (LAH), and the resulting polyols were analysed as their trimethylsilyl ethers by coupled gas chromatography-mass spectrometry. The four major products were assigned the structures (3a), (4a), (5a) and (6a) from their fragmentation patterns.[3]



(3) (4) (5) (6)

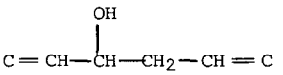
where (a) R = R^1 = H
(b) R = R^1 = D
(c) R = H, R^1 = D

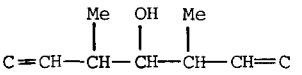
Structures (3a), (4a) and (5a) were confirmed by synthesis from malic acid, diethyl 3-hydroxy-2,4-dimethylglutarate,[4] and diethyl 2-methyl-3-oxoglutarate,[5] respectively. The resulting stereoisomeric mixtures showed glc retention times and mass spectra identical with those of the corresponding degradation products. The structure of the tetrol derivative (6a) was confirmed by conversion to the triol derivative (5a), via acid hydrolysis, oxidation with sodium periodate, reduction with LAH and trimethylsilylation.

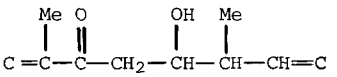
These polyol derivatives (3a), (4a), (5a) and (6a) arise from cleavage of the olefinic bonds in actamycin, but the respective positions in them of the original olefin, carbonyl and hydroxyl carbons are obscured by the hydride reduction process. These positions were defined by deuterium labelling, thus avoiding the need to characterise further new compounds.

Ozonolysis of actamycin followed by reduction with lithium aluminium deuteride (LAD) gave the polyol derivatives (3b), (4b), (5b) and (6b), in which the deuterium atoms, located by mass spectrometry,[3] indicate carbons which were either olefinic or ketonic in actamycin. Finally, reduction of actamycin with LAD and trimethylsilylation of the resulting polyol prior to ozonolysis, followed by reduction of the per-ozonide with LAH, afforded the polyol ethers (3a), (4a), (5c) and (6c). The single deuterium atoms in (5c) and (6c) indicate the position of a carbonyl group in actamycin.

Analysis of these deuterium labelling patterns defines the structural segments (7), (8) and (9) in the parent actamycin.

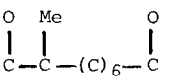
(7) 

(8) 

(9) 

The triol derivatives (5) do not arise from a fourth segment of the ansa bridge, but rather from the same segment (9) as the tetrol derivatives (6) via rearrangement processes during ozonolysis. Ozonides of α,β-unsaturated ketones are known to undergo rearrangement with cleavage of the carbonyl-olefin bond to yield a carboxylic acid.[6] Reduction of this acid to the primary alcohol with LAH or LAD introduces <u>two</u> hydrogen or deuterium atoms at the site of the original carbonyl group, leading to (5a) and (5b), respectively. Similarly, the deuterium-labelled allylic alcohol resulting from initial reduction of segment (9) with LAD undergoes rearrangement on ozonolysis[6] to yield a monodeuterio-aldehyde, which is subsequently reduced with LAH to afford the triol derivative (5c).

In addition to the defined segments (7), (8) and (9), the ansa bridge must also contain a polyenoid segment in order to accommodate the extent of unsaturation known to be present. Catalytic reduction of actamycin over palladium-charcoal, followed by oxidation with chromium trioxide in acetic acid, gave 2-methylazelaic acid, characterised as its dimethyl ester by coupled gas chromatography-mass spectrometry. Its structure follows from major fragment ions at <u>m/z</u> 199 (M^+-OMe), 171 (M^+-CO_2Me), 166 (M^+-2MeOH), 143 (M^+-MeCHCO_2Me), 139 (171-MeOH) and 88 {MeCH=C(OH)OMe, base peak}.[7] This product necessitates a segment (10) in actamycin.

(10) 

The following paper[8] describes evidence from biosynthetically enriched and natural abundance n.m.r. spectra which, in conjunction with the present results, defines the structure (1) for actamycin.

Acknowledgements

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