OLIGOMYCIN: DEGRADATION PRODUCTS AND PART STRUCTURE OF OLIGOMYCIN B[1]

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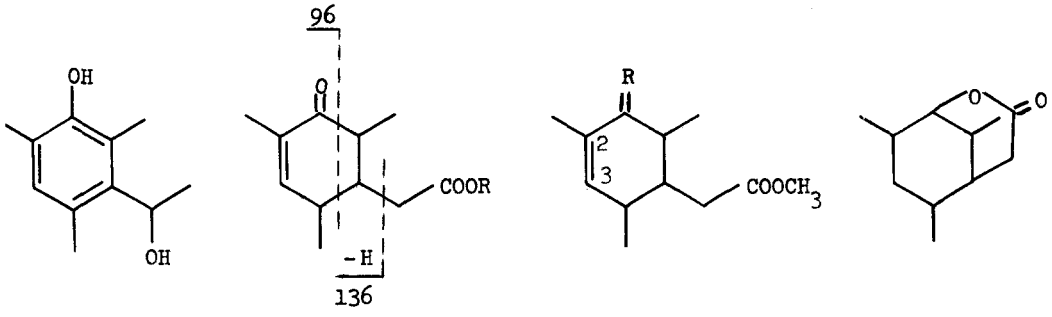
SUMMARY

The part structure 1 has been established for oligomycin B.

Oligomycin, isolated as a crystalline complex of components A, B, and C [2, 3] is an antifungal antibiotic and a potent, specific inhibitor of oxidative phosphorylation. Early chemical work [6] and more recent data [7, 8] show oligomycin B (1) to be $C_{45}H_{72}O_{12}$, MW 804. The compound contains three double bonds [7] in a conjugated diene and a separate unsaturated carbonyl structure (λ_{max}^{MeOH} 218, 224, 233, 242 (sh), 285 (broad) nm; ε 31,700, 32,200, 29,300, 17,700,230). A tetraacetate (MW 972) of 1 and oxidation of hexahydrooligomycin B (2) (MW 810) to a tetradehydro product (MW 802) establish the presence of four secondary hydroxyl groups. Sugar tests were negative, a lactone was suggested by a positive hydroxamic acid test, and acid hydrolysis gave mono- and di-dehydration products.

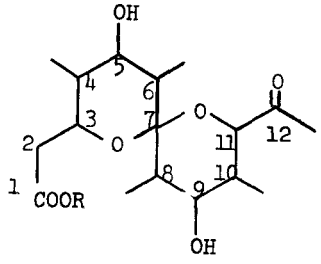
Base hydrolysis (0.05 N methanolic NaOH, 2 hr., 75°) yielded after chromatography, a phenol (3), two acids (4,5), and an aldehyde (6) as major products, plus traces of three higher molecular weight compounds (7,8,9). Structure 3 was proved by spectral data [mass spectrum: m/e 180 (M), 165, 162 (base peak), 147, 137; nmr ($CDCl_3$, 60 mc): δ 1.50 (3H, d, J=7Hz), 2.19, 2.30, 2.37 (singlets, 3 x 3H), 5.32 (1H, q, J=7Hz), and 6.77 (1H, s); λ_{max}^{EtOH} 280 nm, ε 1380], diacetate formation (MW 264), and synthesis from acetylmesitylene of material identical with 3 (IR, UV, MS, NMR, TLC, and GLC comparisons).



3 4 R = H 4a R = CH_3 4b R = H, OH 4c R = O; 2,3-dihydro 4d R = H, OH; 2,3-dihydro 4e

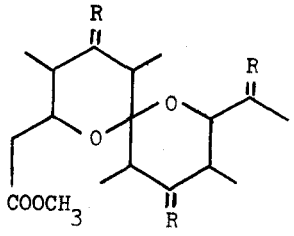
The structure of acid 4 follows from spectroscopic and chemical data obtained for its methyl ester 4a. The mass spectrum of 4a exhibited prominent peaks at m/e 210 (M, $C_{12}H_{18}O_3$)[9], 136 ($C_9H_{12}O$, M-CH_2COOCH_3 + H) and 96 (C_6H_8O); $\nu_{max}^{CHCl_3}$ 1669, 1730 cm^{-1}; λ_{max}^{MeOH} 235 nm (ε 11,034); and nmr ($CDCl_3$, 60 mc), δ 6.4 (broad, 1H), 3.70 (s, 3H), 2.4 (broad singlet, 3H), 1.5 - 1.8 (ca. 5H) and 1.15 (two overlapping doublets, 6H). Reduction of 4a with $NaBH_4$ furnished an ester-alcohol (4b, MW 212) which formed a monoacetate and showed no ultraviolet absorption. Catalytic reduction of 4a gave 4c (MW 212, $\nu_{max}^{CHCl_3}$ 1725 cm^{-1}, broad) which upon $NaBH_4$ treatment furnished 4d (MW 214). On refluxing in methanolic HCl 4d yielded 4e (MW 182) whose infrared carbonyl absorption (1732 cm^{-1}) is in agreement with that expected for a δ-lactone.

Spectroscopic and chemical data allow formulation of structure 5 for the second acid. The corresponding ester 5a exhibited a molecular ion at m/e 358 ($C_{18}H_{30}O_7$) and prominent peaks at m/e 340 (M-H_2O), 297 ($C_{16}H_{25}O_5$, M-H_2O-$COCH_3$), 217 ($C_{10}H_{17}O_5$), 211 ($C_{12}H_{19}O_3$, base), 199 ($C_{11}H_{19}O_3$), 199 ($C_{10}H_{15}O_4$), 187 ($C_9H_{15}O_4$), 155 ($C_9H_{15}O_2$), 137 ($C_9H_{13}O$) and 125 and 124 ($C_8H_{12}O$). Infrared absorption at 3450, 1720 and 1732 cm^{-1} suggested alcohol, ketone and ester functionalities; clearly identifiable resonances in the nmr spectrum ($CDCl_3$, 100 mc) include three-proton singlets at δ 3.72 ($COOCH_3$) and 2.23 ($COCH_3$), a one-proton doublet at δ 4.55 (J = 3 Hz, C-11-H), a complex



5 R = H

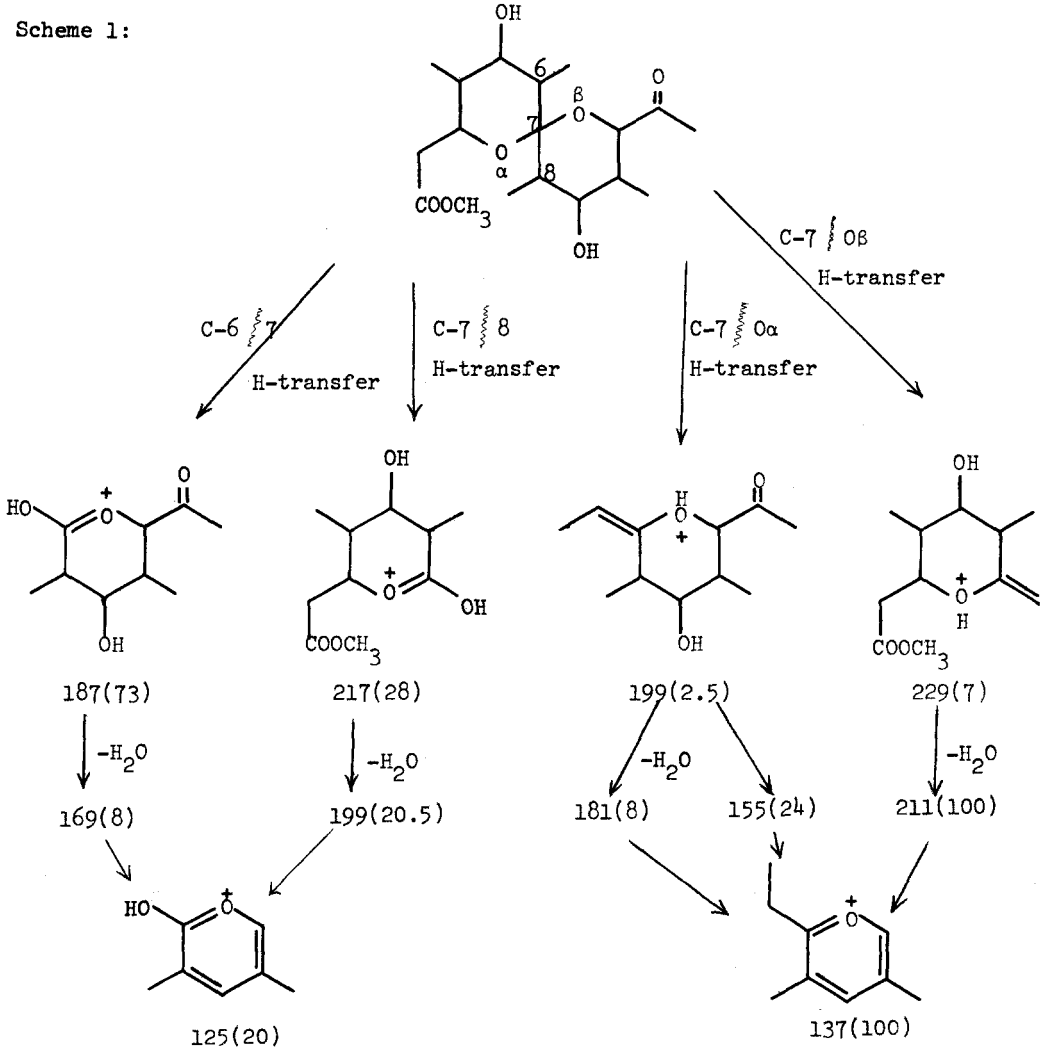
5a R = CH_3



5b R = O

5c R = H,OH

Scheme 1:



multiplet at 3.70 ppm (C-3-H, partially obscured by δ 3.72 resonance) and doublets of four secondary methyl groups (0.88, 7 Hz; 1.04, 6.4 Hz; 1.09, 7.2 Hz; 1.15, 6.6 Hz) in addition to a complex pattern in the region δ 1.6 - 2.4 (ca. 10H). Compound 5a formed a diacetate (MW 442) and could be oxidized to a triketone (5b, MW 354; nmr ($CDCl_3$, 100 mc): δ 3.66 ($COOCH_3$), 2.20 ($COCH_3$), 4.53 (d , 3.2 Hz, C-11-H), 3.88 (multiplet, C-3-H), 2.25 - 3.10 (6H) and the methyl resonances at δ 1.03, 1.08, 1.15, 1.26 (J = 6.5, 6.0, 6.4, 6.7Hz); ν_{max}^{film} 1725 cm^{-1}) which gave no indication of additional hydroxyl functions. Reduction ($NaBH_4$) of 5a yielded 5c (mass spectrum, m/e 360 (M), 217 (base), 211, 201, 199, 189, 171, 137, and 125; ν_{max}^{film} 3475, 1725 cm^{-1}). Mass spectral data on 5, 5a and other derivatives fully support the assigned structure. Major ions arise by the expected cleavages alpha to the ketal oxygen atoms as shown in Scheme 1 (bonds C-6/7, C-7/8, C-7/Oα, C-7/Oβ). Thus initial C-7/Oβ cleavage of 5a, H-transfer, and dehydration lead to the peak at m/e 211 ($C_{12}H_{19}O_3$) whereas the alternative C-7/Oα fragmentation leads to m/e 181 ($C_{11}H_{17}O_2$). Both ions can decompose to the ion of m/e 137 ($C_9H_{13}O$). Rupture of the C-6/7 bond leads to the ion of m/e 187 ($C_9H_{15}O_4$) while initial C-7/8 cleavage furnished the ion of m/e 217. Ions m/e 187 and 217, by loss of appropriate substituents, can decompose to the ions of m/e 169, 199 and 125.

The major product (ca. 50% yield) resulting from base hydrolysis is an aldehyde (6, MW 478, $C_{28}H_{46}O_6$) containing the conjugated diene system [λ_{max}^{MeOH} 225, 232, 240 (sh), 278 nm (ε 28100, 24600, 14800, 88); ν_{max}^{CHCl3} 3460, 2688, 2475, 1724, 1715 cm^{-1}; nmr ($CDCl_3$, 100 mc) δ 9.72 (-CHO) and resonances of an unsubstituted trans diene consisting of a pattern of two quartets centered at δ 5.95 (H_B, H_C; $J_{AB} = J_{CD}$ = 15 Hz, J_{BC} = 10 Hz) and a complex pattern at δ 5.0 - 5.7 (H_A, H_D)]. The aldehyde function of 6 (as well as a keto function contained within the $C_{14}H_{23}O_4$ residue) could be reduced ($NaBH_4$) to an alcohol (6a) with a very similar ultraviolet chromophore (λ_{max}^{MeOH} 226,

231, 240 (sh), 277 nm (ε 22650, 24000, 12400, 195[10]). Catalytic reduction, however, furnished a hexahydro derivative (6b),(MW 484; ν_{max} 3448, 1730 cm^{-1}) with only a residual ultraviolet maximum of 283 nm (ε 72) due a ketone functionality in the $C_{14}H_{23}O_4$ moiety . Both 6a and 6b exhibited a doublet at δ 3.45 (2H, J = 6 Hz) due to the new primary alcohol function $CHCH_2OH$; oxidation of 6 or 6b yielded the corresponding unsaturated or saturated keto acid. From ozonolysis of 6 the aldehydes 6c [MW 384, $C_{21}H_{36}O_6$; ν_{max} 3448, 2717, 2496, 1724 cm^{-1}; nmr ($CDCl_3$, 60 mc) δ 9.75 (-CHO)] and 6d [MW 410, $C_{23}H_{38}O_6$; ν_{max}^{film} 3450, 2750, 1730, 1690 cm^{-1}; λ_{max}^{EtOH} 223, 296 nm (ε 12950, 160); nmr ($CDCl_3$, 60 mc) δ 9.4 (H_A, d, J_{AB} = 7.6 Hz), 6.2 (H_B, J_{AB} = 7.6 Hz, J_{BC} = 16 Hz) and 6.6 (H_C, J_{BC} = 16 Hz, J_{CD} = 7.6 Hz)] were obtained. Compound 6d could be reduced (H_2/PtO_2) to 6e (MW 414; ν_{max}^{film} 3350, 1725 cm^{-1}) lacking low wavelength ultraviolet absorption. Baeyer-Villiger reaction on 6c followed by base hydrolysis furnished 6f [MW 372, $C_{20}H_{36}O_6$; $\nu_{max}^{CHCl_3}$ 3390, 1727 cm^{-1}, λ_{max}^{MeOH} 285 nm (ε 39)]. The mass spectrum of the trimethylsilyl-ether derivative of 6f showed an intense peak at m/e 131 (CH_3CH_2-CH = $\overset{+}{O}$-Si$(CH_3)_3$) not observed in the spectra of the silyl ether derivatives of 6 a,b,c,d suggesting a hydroxypropyl functionality. A clearly identifiable methyl triplet (δ 1.10, J = 7.1 Hz) in the nmr spectrum of the triketone obtained by CrO_3 oxidation of 6f supports this assignment. Direct ozonolysis of oligomycin B (1) gave a mixture of esters 6g and 6h (MW 440, 442 respectively), which upon Baeyer-Villiger reaction and base hydrolysis furnished 6f. The isolation of the glycolate and glyoxylate esters (6g, 6h) confirms an α,β-unsaturated lactone for oligomycin B. A secondary methyl group alpha to the hydroxyl function involved in lactone formation as shown in structures 6-6h is supported by analysis (spin decoupling) of nmr spectra of 6, 6a, 6f, 6h, and the acetates of 6 and 6f. Since our data do not suffice as yet for a complete description of the structure of compound 6, detailed discussion of all results appears unwarranted and only data directly relevant to part structure 6 has been considered here.

6 R_1 = CHO, R_2 = $C_{14}H_{23}O_4$

6a R_1 = CH_2OH, R_2 = $C_{14}H_{25}O_4$

6b

6c R_1 = CHO, R_2 = H

6d R_1 = , R_2 = H

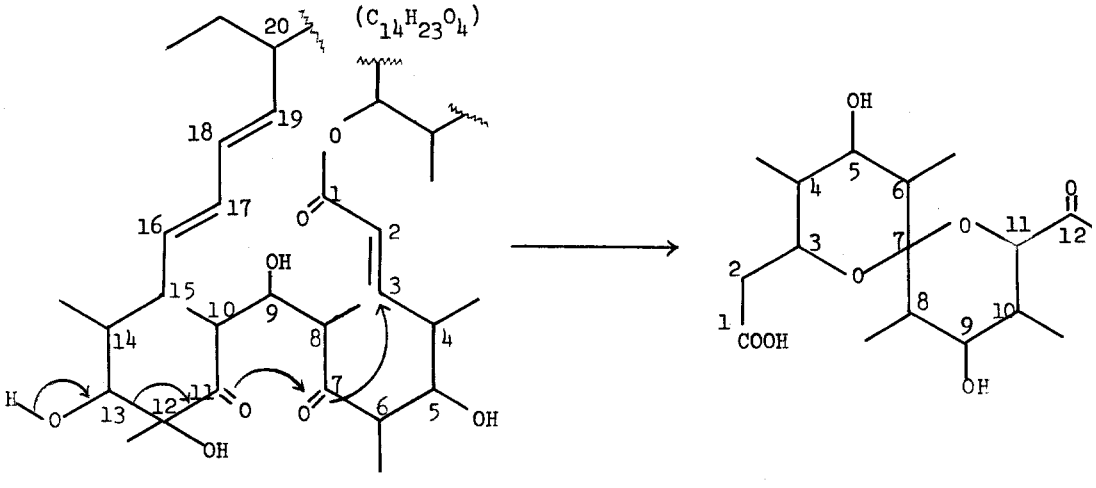
6e R_1 = $-CH_2CH_2CH_2OH$, R_2 = H

6f R_1 = OH, R_2 = H

6g R_1 = CHO, R_2 = COCHO

6h R_1 = CHO, R_2 = $COCH_2OH$

Part structure 1 for oligomycin B readily explains the formation of aldehyde 6, phenol 3 and acids 4 and 5 upon base treatment. For example, phenol 3 derives from carbon chain $C_6 \rightarrow C_{12}$ by retro-aldol reactions and aldol condensation. Acid 4 results from retro-aldol cleavage of the C-8/9 bond, Michael addition of C-8 to the C-2,3 double bond, and hydrolysis. Acid 5 represents the entire chain from C-1 to C-12; its genesis is indicated by the arrows in structure 1. Aldehyde 6 then simply represents the remainder of the molecule. Structure 1 is in agreement also with the isolation of several other minor degradation products from the base hydrolysis mixture.



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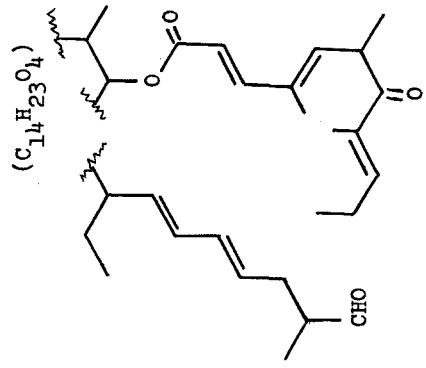
Compounds 7 (MW 588, $C_{34}H_{52}O_8$) and 8 (MW 656) were not completely characterized, but their mass spectral fragmentation pattern (e.g. loss of the respective ester chain) and chemical transformations (e.g. hydrogenation to decahydroderivatives, hydrolysis of 8 to 6) support these assignments. In addition compound 9 (MW 696) was isolated, to which the structure shown can be assigned based on its molecular weight and mass spectral fragmentation pattern, although it may well exist in isomeric, internally cyclyzed form. The possible genesis of 7, 8, and 9 from structure 1 is self-evident.



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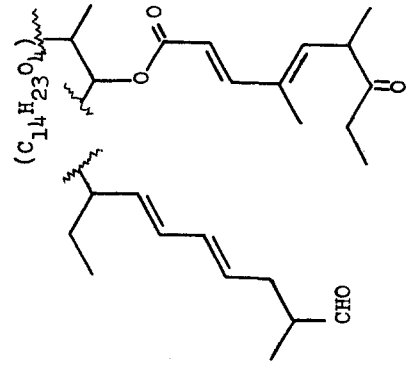

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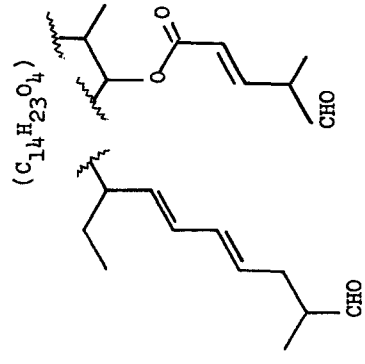

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The mass spectrum of oligomycin B (1) likewise supports the structure. The spectrum shows prominent peaks at m/e 702, 674, 656 (674-H_2O) and 588 which correspond to fragments arising by all the possible electron-impact induced retro-aldol type reactions (e.g. m/e 702 represents loss of C-10 → C-12 unit by double retro-aldol; 674 = loss of C-9 → C-12 unit, etc). An intense peak at m/e 460 corresponds to a fragment of structure 6-H_2O.

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9. All compositions for molecular or fragment ions (except for 1) given in this paper were determined by high resolution mass spectrometry unless otherwise indicated. We are indebted to Dr. A. L. Burlingame (University of California at Berkeley) for the use of his equipment and to Miss Martha Petrie for determining many of the high resolution spectra.
10. The compound shows no carbonylabsorption in the infrared spectrum. We attribute the high extinction at 277 nm to an impurity.