2.0 (br mult, H-4), 1.79 (br mult H-4′); mass spectrum (CI, methane), m/z (fragment, %) 159 (M^+ + 1, 52), 158 (M^+, 15), 127 (M^+ − OMe, 20), 98 (M^+ − H − CO_2Me, 16), 85 (M^+ − CH_2CO_2Me, 100); IR (neat) 1770, 1750 cm^{-1}; UV (MeOH λ_{max} 205 nm.

Xestin A (5). Reverse-phase flash column chromatography over 50–100 μm ODS followed by reverse-phase HPLC (10 μm ODS, 92:08 MeOH–H_2O as eluent) yielded pure **5**, whose retention time was longer than that of **6**. Physical properties were as follows: mp (MeOH) 55–56 °C; $[\alpha]^{20}_D$ +26.5° (c 0.37, CH_2Cl_2); NMR (assignments by ^{13}C NMR APT and INEPT, ^1H-^1H COSY); ^{13}C NMR (75 MHz, $CDCl_3$) 170.1 (C-1), 132.3 (C-20), 131.8 (C-21 or C-22), 130.5 (C-22 or C-21), 130.3 (C-4), 127.0 (C-23), 126.7 (C-5), 101.2 (C-6), 73.6 (C-3), 52.1 (ester OMe), 51.4 (ketal OMe), 36.4 (C-2), 34.9 (C-19), 32.6 (C-7), 29.6 (10 CH_2) 23.4 (C-8), 18.1 (C-24) ppm; ^1H NMR (300.1 MHz, $CDCl_3$) δ 6.11 (dd; J = 1.2, 10.2 Hz, H-4), 6.00 (m, H-21, H-22), 5.84 (dd, J = 2.1, 10.2 Hz, H-5), 5.56 (m, H-20, H-23), 5.00 (m, H-3), 3.72 (s, ester OMe), 3.38 (s, ketal OMe), 2.61 (dd, J = 7.5, 16.2 Hz, H-2), 2.50 (dd, J = 6.6, 15.9 Hz, H-2′), 2.03 (4-line mult, H-19–19′), 1.72 (d, J = 6.3 Hz., Me-24), 1.64 (m, 4 H), 1.2–1.4 (10 CH_2); ^1H–^1H COSY (selected correlations), H-2 to H-2′, H-2-2′ to H-3, H-3 to H-4 and H-5, H-4 to H-5, Me to conjugated double bonds, double bonds to H-19–19′, H-19–19′ to long chain; CI (methane) mass spectrum, m/z (fragment, %) 437 (M^+ + 1, 20), 436 (M^+, 5), 405 (M + 1 − O_2, 8), 377 (M^+ − MeOCO, 15), 359 (M^+ − MeOCO − H_2O, 10), 345 (M^+ − O_2 − CH_3OCO, 10), 317 (M^+ + 1 − $MeOCOCH_2$ −O_2 −Me, 45), 289 (18), 171 (100), 157 (40); IR (neat) 1740 cm^{-1}; UV (MeOH) λ_{max} 227 nm (ε 29 000).

Xestin B (6). Purification was as described above for compound **5**. Physical properties were as follows: $[\alpha]^{20}_D$ +19.61° (c 0.11, Cl_2CH_2); ^{13}C NMR (25.1 MHz, $CDCl_3$) 170.7 (C-1), 132.0 (C-20, and C-21 or C-22), 130.4 (C-22 or C-21), 129.4 (C-4), 127.1 (C-23), 126.5 (C-5), 100.7 (C-6), 73.4 (C-3), 51.7 (ester OMe), 51.1 (ketal OMe), 37.3 (C-2), 34.8 (C-19), 32.6 (C-7), 29.5 (10 CH_2), 23.5 (C-8), 17.8 (C-24) ppm; ^1H NMR (300.1 MHz, $CDCl_3$) δ 6.17 (dd, J = 4.5, 10.2 Hz, H-4), 5.97 (m, H-21, H-22), 5.85 (dd, J = 1.5, 9.9 Hz, H-5), 5.56 (m, H-20, H-23), 4.78 (m, H-3), 3.70 (s, ester OMe), 3.37 (s, ketal OMe), 2.91 (dd, J = 8.7, 15.9 Hz, H-2), 2.58 (dd, J = 9.0, 16.5 Hz, H-2′), 2.01 (4-line mult, H-19–19′), 1.70 (d, J = 6.3 Hz, Me-24), 1.62 (m, 4 H), 1.2–1.4 (10 CH_2); CI (methane) mass spectrum; m/z (fragment, %) 437 (M^+ + 1, 15), 436 (M^+, 5), 405 (M^+ + 1 − O_2, 15), 377 (M^+ − MeOCO, 10), 373 (M^+ − O_2 − MeO, 15), 359 (M^+ − MeOCO − H_2O, 45), 345 (M^+ − Me − O_2 − MeOCO, 70), 317 (M^+ + 1 − $MeOCOCH_2$ − O_2 − Me, 30), 289 (20), 171 (70), 157 (100); IR (neat) 1740 cm^{-1}; UV (MeOH) λ_{max} 227 nm (ε 29 000).

LAH Reduction of Xestin A (5) to Alcohol 7. $LiAlH_4$ (2 mg, 0.052 mmol) was dissolved in 1 mL of dry THF with 3 μL of absolute EtOH (0.052 mmol). This mixture was added to a stirred solution, blanked with nitrogen, of xestin A (21 mg, 0.048 mmol) dissolved in 2 mL of dry THF. After 1.25 h workup commenced by addition of water (3 mL) and then extraction with ethyl ether (4 × 5 mL) and CH_2Cl_2 (1 × 5 mL). The organic layers were combined, dried over $MgSO_4$, and evaporated under low pressure, giving pure **7** (20.58 mg). Its spectroscopic properties were as follows: ^{13}C NMR (25.1 MHz, $CDCl_3$) 132.2 (C-20), 131.9 (C-21 or C-22), 131.5 (C-22 or C-21), 130.4 (C-4), 126.5 (C-5 and C-23), 101.1 (C-6), 75.4 (C-3), 59.1 (C-1), 51.2 (MeO), 35.2 (C-2), 34.9 (C-19), 32.5 (C-7), 29.3 (10 CH_2), 23.4 (C-8), 17.9 (C-24) ppm; ^1H NMR (300.1 MHz, $CDCl_3$) 6.06 (dd, J = 1.2, 10.2 Hz, H-4), 6.00 (m, H-21, H-22), 5.84 (dd, J = 2.1, 10.2 Hz, H-5), 5.56 (m, H-20, H-23), 4.80 (m, H-3), 4.12 (m, H-1), 3.77 (m, H-1′, overlapped with OH), 3.39 (s, MeO), 2.03 (m, H-19–19′), 1.72 (d, J = 6.3 Hz, Me-24), 1.2–2.0 (13 CH_2); CI (methane) mass spectrum, m/z (fragment, %) 391 (M + 1 − H_2O, 15), 363 (M^+ − C_2H_4OH, 25), 348 (363 − Me, 75), 331 (363 − MeOH, 65); IR (neat) 3200–3600 cm^{-1} (OH); UV (MeOH) λ_{max} 227 nm (ε 29 000).

Hydrogenation of Xestin A (5) to Compound 8. Xestin A (5 mg) was dissolved in 5 mL of ethyl acetate with 5 mg of Na_2CO_3 and a catalytic amount of Pd/C 10%. Hydrogenation proceeded for 3 h (1 atm of pressure). The reaction mixture was filtered and the solvent evaporated under low pressure, affording after purification **8** (4.45 mg): $[\alpha]^{20}_D$ −10.9° (c 0.036, $CHCl_3$); ^{13}C NMR (25.1 MHz, $CDCl_3$) 210.9 (C-6), 172.9 (C-1), 67.4 (C-3), 51.6 (ester OMe), 42.8 (C-2 or C-7), 41.4 (C-7 or C-2), 38.6 (C-4 or C-5), 36.0 (C-5 or C-4), 31.8 (C-22), 30.2 and 29.1 (13CH_2), 23.8 (C-8), 22.6 (C-23), 13.9 (C-24) ppm; ^1H NMR (300.1 MHz, $CDCl_3$) δ 3.98 (m, H-3), 3.70 (s, ester OMe) 2.59 (m, H-5–5′), 2.45 (m, H-2–2′ partially overlapped with H-7–7′), 2.41 (m, H-7–7′), 1.70 (m, H-4–4′), 1.55 (m, H-8–8′), 1.4–1.1 (15 CH_2), 0.87 (br t, Me-24); A ^1H–^1H COSY spectrum was consistent with the proposed structure; H-2–2′ to H-3, H-3 to H-4-4′, H-4-4′ to H-5-5′, H-7-7′ to H-8-8′, H-8–8′ to $(CH_2)_{15}$, Me-24 to $(CH_2)_{15}$; CI (methane) mass spectrum, m/z (fragment, %) 413 (M^+ + 1, 10), 412 (M^+, 5), 397 (M^+ − Me, 40), 395 (M^+ − 17, 80), 381 (M^+ − MeO, 100), 367 (85), 253 ($C_{18}H_{37}$, 10); IR (neat), 3600–3200 (OH), 1748 (ester), 1710 (ketone) cm^{-1}.

Hydrogenation of Xestin B (6) to Compound 8. Xestin B (5 mg) was dissolved in 5 mL of ethyl acetate with 5 mg of Na_2CO_3 and a catalytic amount of Pd/C 10%. Hydrogenation proceeded for 3 h (1 atm of pressure). The reaction mixture was filtered and the solvent evaporated under low pressure, affording after purification **8** (3.9 mg), $[\alpha]^{20}_D$ −14.0° (c 0.021, $CHCl_3$). All spectral properties were identical with the other sample of **8**.

Chiral Shift Reagent Study of 8. With ^1H NMR as a monitor, each sample of **8**, obtained as described above, was titrated with tris[((3,4,5,6,7,8,9-heptafluoropropyl)hydroxymethylene)-(+)-camphorato]europium (III), Eu(hfc)$_3$. Identical concentrations of **8** (6.3 mM) in $CDCl_3$ were prepared, and the shift of the OMe was observed as Eu(hfc)$_3$ was added. Identical incremental OMe shifts were observed for both samples as the shift reagent concentration was increased (δ values for OMe relative to Me_4Si): [Eu(hfc)$_3$] = 0 mM, δ 3.70; [Eu(hfc)$_3$] = 0.3 mM, δ 3.70; [Eu(hfc)$_3$] = 3.0 mM, δ 4.30; [Eu(hfc)$_3$] = 10 mM, δ 5.0.

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Registry No. 1, 104532-57-2; 2, 63545-49-3; 5, 104532-58-3; 6, 104597-39-9; 7, 104532-59-4; 8, 104532-60-7.

Structure Determination of Oligomycins A and C[1a]

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The structures of oligomycins A (2) and C (3) were established by chemical correlation of their respective degradation products with those derived from oligomycin B, whose structure is known. The mass spectral fragmentation behavior of the spiroketal under electron impact conditions is discussed.

The oligomycin antibiotic complex was first isolated in 1954[2] from a strain of *Streptomyces diastatochromogenes*. The complex, consisting of variable proportions of three major components A, B, and C[3]—depending on the strain

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Table I. Selected Physical Properties of the Oligomycins and Rutamycin

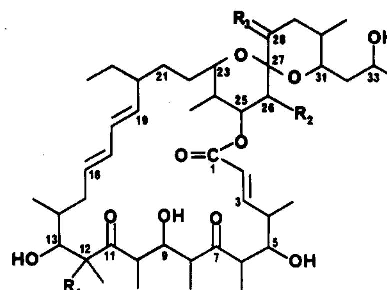
	oligomycin[a] A	oligomycin[a] B	oligomycin[a] C	rutamycin[b]
mp, °C	150–151	160–161	198–200	127–128
$[\alpha]_D$, deg	−54.5 (*c* 4.40 diox)	−46.4 (*c* 0.76 diox)	−80.7 (*c* 3.70 diox)	−40.3 (*c* 4.4 diox)
mol form	$C_{45}H_{74}O_{11}$[c]	$C_{45}H_{72}O_{12}$[c]	$C_{45}H_{74}O_{10}$[d]	$C_{44}H_{72}O_{11}$
UV λ_{max} (MeOH) (nm) (ϵ)	275 (130)	285 (230)	275 (145)	
	242 (18 500)	242 (17 700)	239 (16 900)	
	232 (32 900)	233 (29 300)	231 (29 200)	230
	225 (37 400)	224 (32 200)	224 (33 500)	225 (34 500)
	220 (34 000)	218 (31 700)	219 (32 700)	
IR ν_{max} (cm^{-1})	3440–3500	3446–3500	3500	3448
	1700	1712	1700	1700
	1638	1690	1640	1640
		1640		

[a] Reference 9. [b] Reference 12. [c] Prouty, W. F.; Schnoes, H. K.; Strong, F. M. *Biochem. Biophys. Res. Commun.* **1969**, *34*, 511–515. [d] Prouty, W. F. Ph.D. Thesis, University of Wisconsin, Madison, Wisconsin, 1970.

of organism[4] and cultural conditions[5]—was characterized by broad-spectrum antifungal activity. Due to their toxicity these compounds are not clinically useful; however, they are of considerable scientific interest as ATPase inhibitors and are commercially available. The use of oligomycin in experimental work, pioneered by Lardy,[6] has contributed substantially to our knowledge of oxidative phosphorylation processes.[7,8]

Selected physicochemical data on the oligomycins and rutamycin are presented in Table I. The oligomycins are neutral macrolide antibiotics characterized by UV absorption maxima at approximately 225 and 232 nm and a shoulder at about 240 nm.[9] These compounds contain a 26-membered α,β-unsaturated lactone with a conjugated diene fused to a bicyclic spiroketal. Oligomycin B (**1**) was the first of this family to have its structure determined. In 1971 a partial structure including carbons 1–20, 24, and 25 based upon chemical degradation and spectroscopic data was published.[10] An X-ray crystallographic study[11] verified the earlier conclusions and solved the remainder of the structure including the elusive bicyclic spiroketal.

Other antibiotics in the oligomycin family include rutamycin,[12] hondamycin,[13] botrycidin,[14] and possibly peliomycin.[15] Structure **4** was proposed for rutamycin on the basis of spectroscopic data[16] and was later confirmed by X-ray crystallography.[17] Hondamycin was shown to



be chromatographically distinct from oligomycins A, B, and C;[13] however, no structure was proposed. Recently, the identity of botrycidin with rutamycin was established and a new compound, rutamycin B (**5**), was identified.[18] The rutamycin B structure was determined by application of base degradation procedures first used by Chamberlin[19] and extended by Prouty.[10] In view of recent developments, including efforts directed toward screening for ATPase inhibitors,[20] it seemed timely to report this work concerning the chemistry and structures of oligomycins A and C.

The structures of oligomycins A (**2**) and C (**3**) were assigned by correlating their respective base degradation products with those derived from oligomycin B. Upon reflux in dilute methanolic base, oligomycin B affords compounds **6**, **7**, **9**, and **10**.[10] Aldehyde **6** and acid **7** arise through initial retroaldol fragmentation of the 12,13 bond forming the aldehyde group at 13 and forming an enolate

(1) (a) This work is taken in part from Ph.D. Thesis research done in the Biochemistry Department of the University of Wisconsin—Madison under the direction of Professor H. K. Schnoes. A preliminary account of this work was presented at the 169th National Meeting of the American Chemical Society, Philadelphia, PA, April 6–11, 1975; ORGN 72. (b) Present address: Medical Research Division, American Cyanamid Company, Lederle Laboratories, Pearl River, NY 10965.

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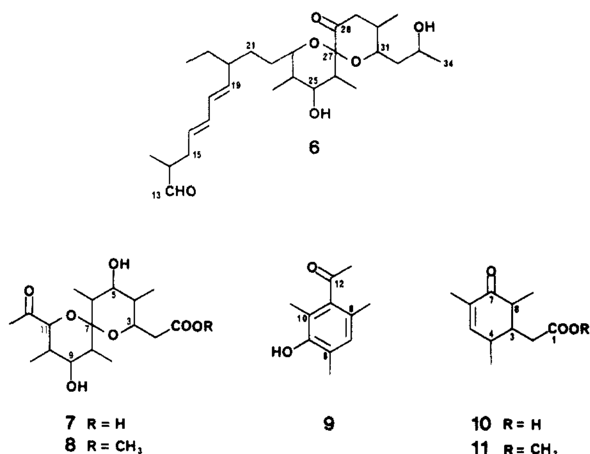


ion between carbons 11 and 12. The spiroketal of **7** arises by attack of the enolate through oxygen 11 on the 7 ketone with subsequent Michael addition of the resulting oxygen anion to the α,β-unsaturated ester. Hydrolysis of the ester liberates the aldehyde **6** and acid **7**. Compounds **9** and **10** are derived from partially overlapping segments of the 1–12 portion of the chain via retroaldol cleavage, cyclization, and dehydration. Phenol **9** is formed by addition of the 6 carbanion resulting from retroaldol fragmentation of the 5,6 bond to the 11 ketone. Elimination of two molecules of water and enolization of the 9 ketone yields the phenolic moiety which is released upon fragmentation of the 12,13 bond. The six-membered ring of **10** arises by Michael addition of the 8 carbanion, formed by retroaldol cleavage of the 8,9 bond, to the α,β-unsaturated ester. Saponification and elimination of a molecule of water yields acid **10**.

Aldehyde **6** is obtained in reasonable yield (50%) and contains carbons 13–34 of the molecule including the diene and spiroketal system. Only trace quantities of compounds **7**, **9**, and **10** are formed in the base degradation; however, they are highly characteristic of the oligomycin carbon chain from 1–12.

Oligomycin A. As indicated in Table I, oligomycin A contains one less oxygen and two more hydrogen atoms than oligomycin B. These differences could result from the deoxygenation of a carbonyl group in oligomycin B to a methylene group in oligomycin A. Evidence to support this hypothesis is found in a comparison of spectral data between the two compounds. The IR spectrum of oligomycin A lacks the shoulder absorption at 1712 cm^{-1} found in the spectrum of B. This absorption is also lacking in the spectrum of rutamycin, suggesting that it is due to the 28 keto function. Similarly, the 203.0-ppm signal in the ^{13}C NMR spectrum of oligomycin B (Table II), which has been assigned to C-28, is absent in the spectrum of A.[21] Given the otherwise very similar nature of the two compounds, the most straightforward interpretation of these data is that oligomycin A is represented by structure **2**, i.e., 28-deoxy oligomycin B.

Basic degradation of oligomycin A yields acids **7** and **10** plus aldehyde **12**, whose structure was determined by the sequence of reactions illustrated in Scheme I. The aldehyde was first reduced by $NaBH_4$ to give the primary alcohol **13** which was converted to the tris(*p*-bromobenzoyl ester) **14**. Partial ozonolysis of the diene gave aldehydes **15** and **16**. The structure of **15** follows from analysis of

(21) While this manuscript was in preparation, we became aware of a report concerning the complete assignment of the ^{13}C NMR signals for oligomycin A [Morris, G. A.; Richards, M. S. *Magn. Reson. Chem.* **1985**, *23*, 676–683.] which is in accord with the assignments made in this paper.

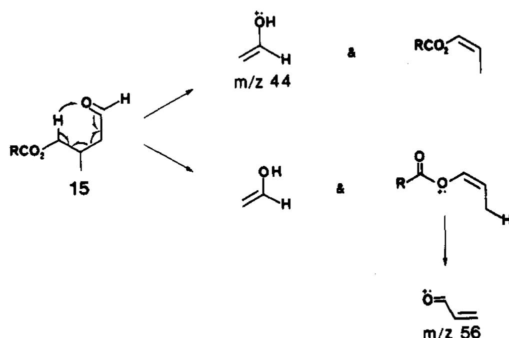
Table II. ^{13}C NMR Chemical Shifts[a] for the Oligomycins and Rutamycin

oligomycin A	oligomycin B	oligomycin C	rutamycin
220.1	220.2	221.6	220.0[b]
219.9	219.8	215.9	165.0
165.1	203.0	165.1	148.6
148.5	165.1	148.2	137.6
137.7	148.8	137.5	132.5
132.4	137.0	132.2	130.4
130.3	132.1	130.4	129.5
129.4	130.6	129.5	122.6
122.7	129.8	122.8	97.39
99.18	122.8	99.11	83.09
83.00	100.0	76.43	73.11
76.26	82.95	72.85	72.70[b]
73.02	75.74	71.29	70.95
72.75	72.90	70.77	69.74
72.48	72.64	68.95	67.58
69.04	72.03	67.26	64.75
67.29	71.02	64.60	46.67
64.73	67.11	49.48	46.00
46.52	64.48	48.94	45.73
45.98[b]	46.00[c]	47.44	42.62
42.61	43.98	45.95	42.08
42.14	41.89[b]	45.62	40.20
40.25	40.20	42.63	38.58
38.50	38.31	40.16	35.48[b]
37.76	36.90	37.76	33.59
35.87	35.68	37.43	31.29
33.58	32.60	35.74	30.62[b]
31.49	31.10[c]	33.40	29.81
31.02	28.60	31.39	28.46
30.54	24.90	30.94	26.44
28.52	21.05	30.48	24.68
26.57	17.82	28.46	21.18
26.03	14.44	26.58	17.80
24.74	13.91	26.00	14.59
21.17	12.69	24.76	13.89
17.94	12.02	17.94	12.00
14.50	11.55	13.58	11.33
14.09	9.25	13.26	9.58
12.07	8.32	12.74	8.36
11.80	5.82	12.02	5.26
11.26		11.76	
9.37		11.24	
8.36		9.55	
6.07		8.19	
		5.78	

[a] $CDCl_3$ solutions, ppm downfield from Me_4Si. [b] Two superimposed signals. [c] Three superimposed signals.

spectroscopic data. Aldehyde and ester functions are supported by the IR data: 2710, 2810, 1723 cm^{-1} and 1112, 1269, 1723 cm^{-1}, respectively. The ^1H NMR spectrum contains a narrow triplet at δ 9.80 indicative of an aldehyde adjacent to a methylene group, two complex two-proton absorptions resulting from diastereotopic methylene groups (δ 2.3–2.7, 15-CH_2; δ4.0–4.4, 13-CH_2), and a methyl doublet (δ 1.11, J = 6.6 Hz). The signal for H-14 was not fully resolved. Molecular ions in the mass spectrum at m/z 284, 286 are indicative of a bromobenzoate derivative of a compound of 102 daltons. Bromine-containing ions at m/z 256, 258, and 241, 243 arise by expulsion of CO from the aldehyde and loss of CH_2CHO, respectively. Other structurally characteristic ions include an ion at m/z 101 resulting from cleavage α to the benzoyl ester and abundant ions at m/z 44 and 56. These latter ions are derived through the fragmentation process illustrated below, which is initiated by a McLafferty rearrangement to give the m/z 44 ion or, if the charge is retained by the olefinic product, the ion at m/z 56. These data define **15** and thereby establish the structure of the 13–16 portion of oligomycin A.

The companion aldehyde **16** from the selective ozonolytic cleavage of the 16,17 bond has a characteristic ^1H



NMR spectrum clearly defining the unit 17–20. The aldehyde proton (H-17) signal appears at δ 9.38 as a doublet (J = 7.2 Hz) coupled to H-18 (δ 5.91, dd, J = 7.2, 15.5 Hz). The H-19 resonance at δ 6.24 is also a doublet of doublets (J = 7.9, 15.5 Hz), indicating trans olefinic geometry and bonding to a methine carbon at C-20. The remaining portion of the structure of **16** was established by conversion to **19**, a compound unambiguously derived from oligomycin B. Compound **16** was first converted to the primary alcohol with $NaBH_4$ and saponified, then treated with ozone, reduced, and acetylated to give **19**.

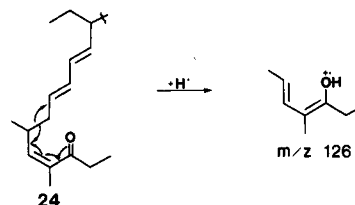
Production of **19** from oligomycin B was accomplished by deoxygenation of the 28 ketone of compound **20** obtained by ozonolysis of the intact antibiotic. The 28 ketone proved to be unreactive, presumably because of steric hindrance, and no results were obtained by using the Wolff–Kishner reaction or several of its modifications. The conversion was accomplished through the formation of the tosylhydrazone derivative of **21** under forcing conditions, followed by elimination with lithium hydride to the olefin **23** and catalytic reduction to the desired product. Attempts to reduce **22** directly with sodium cyanoborohydride[22] or sodium borohydride[23] were unsuccessful. The identity of the product derived from oligomycin B with that from **12** was established by TLC, GC, and mass spectrometry.

Mass Spectrometric Fragmentations of the Spiroketal System. Although the structures of **12** and **19** were formally proven through chemical interconversions, these compounds and numerous derivatives were studied extensively by electron impact mass spectrometry (EIMS). The bicyclic spiroketal system of the oligomycins undergoes a series of highly characteristic fragmentation processes under EIMS conditions. The most important of these are shown in Scheme II and the data are presented in Table III. Generally, these ions result from cleavage of one carbon–oxygen bond and one carbon–carbon bond within a given ring. Fragments of type a arise by cleavage of 31,Oa and 27,28 with hydrogen transfer. Ions b and c are formed by scission of 27,Oa plus 28,29 and 30,31, respectively. Analogous fragmentations within the other ring (23–27) yield ions e, f, and g. Ion d is formed by a retro-Diels–Alder process following elimination of HOR_2. Given this set of relatively abundant fragment ions, it has been possible to characterize degradation products on a microgram scale.

Reassembly of products **8** and **12** into a single chain according to the oligomycin B precedent establishes the gross structure of oligomycin A; it remains only to determine the point of closure of the lactone ring, either at the 25 or 33 position. The signal assigned to the carbinyl proton in the ^{1}H NMR spectrum of oligomycin A is a doublet of doublets at δ 4.83 with coupling constants of 4.8 and 11.4 Hz. This pattern is only reasonable for H-25 in an axial conformation, coupled to adjacent equatorial (4.8 Hz) and axial (11.4 Hz) protons. Taken together, these data complete the proof of structure **2** for oligomycin A.

Oligomycin C. Oligomycin C contains one less oxygen atom than does the A component. As was found for oligomycin A and rutamycin, two ketone carbons are indicated by the ^{13}C NMR data (221.6, 215.9 ppm), neither corresponding to the 28 ketone of oligomycin B. The other conspicuous difference in the ^{13}C NMR data is the absence of the absorption at 83 ppm, assigned to the tertiary alcohol carbon at position 12, found in the spectra of the other antibiotics. This observation suggests that in oligomycin C, C-12 is not oxygenated. On the basis of these premises, oligomycin C can be represented by structure **3**, i.e., 12-deoxyoligomycin A.

Oligomycin C also gives compounds **10** and **12** upon base degradation. Acid **7** was not isolated; instead a novel α,β-unsaturated ketone **24** was obtained, the formation of which is linked to the absence of the tertiary hydroxyl group at position 12. The structure of **24** was largely deduced from spectroscopic data. The α,β-unsaturated ketone chromophore is indicated both by the UV absorption data, which show similar maxima to those of **12** but with greatly enhanced extinctions (225 nm, $\epsilon = 5.6 \times 10^4$; 230 nm, $\epsilon = 5.5 \times 10^4$; and 242 nm (sh), $\epsilon = 3.5 \times 10^4$) and by the presence of a characteristic carbonyl band in the IR spectrum at 1671 cm^{-1}. The ^{1}H NMR spectrum, while similar to that of **15**, contains some significant additions. A broad one-proton doublet at δ 6.36, J = 9.5 Hz, is attributed to the β olefinic proton of a conjugated ketone. Methyl substitution at the α olefinic position is supported by a signal at δ 1.77 (d, J = 1.0 Hz) showing allylic coupling. Ethyl substitution of the ketone is indicated by the methylene quartet at δ 2.66, J = 7.3 Hz. The EIMS of **24** contains a molecular ion at m/z 532 plus a series of fragment ions characteristic of the bicyclic spiroketal (i.e., m/z 167, 184,185, 213, 225; Scheme II, Table III). These latter ions and the ions of m/z 407 and 389, which result from cleavage of the 14,15 bond, suggest the carbon skeleton between 15–34 is identical with that of **12**. The remainder of the molecule is represented by the base peak in the spectrum at m/z 126 which was shown by high resolution measurements to consist of $C_8H_{14}O$. This highly stabilized ion is formed by cleavage of the 14,15 bond with proton transfer to the carbonyl oxygen as shown below.[24] On the basis of these data, the structure of **24** was proposed as shown.

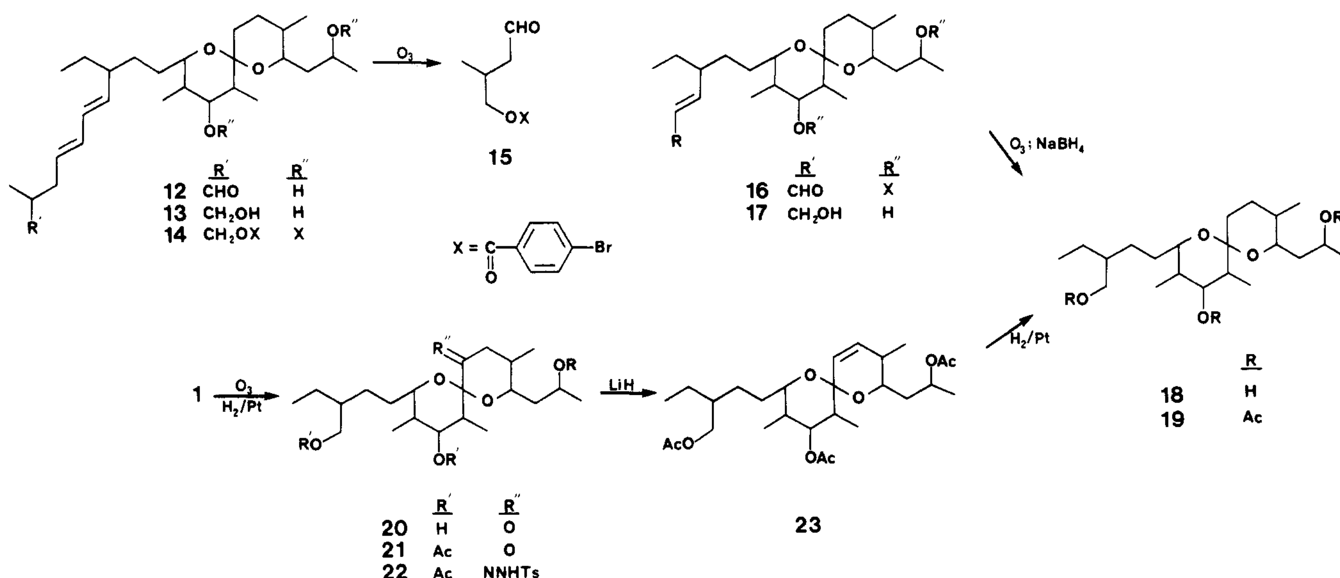


Additional evidence for the structure was obtained by conversion of **24** into saturated aldehyde **26**, whose identity was assured by production from **12** as shown in Scheme III. Intermediate **25** was prepared by selective homogeneous catalytic hydrogenation of the diene, leaving the trisubstituted olefin intact. Product **25** was characterized

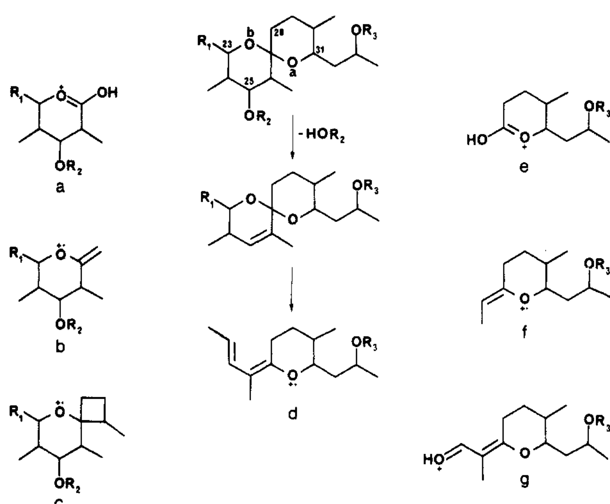
(22) Hutchins, R. O.; Maryanoff, B. E.; Milewski, C. A. *J. Am. Chem. Soc.* **1971**, *93*, 1793–1794.
(23) White, J. D.; Gupta, D. N. *J. Am. Chem. Soc.* **1968**, *90*, 6171–6177.

(24) Interestingly the tetrahydro derivative **25** (Scheme III) does not show this rearrangement ion. Instead, the ion resulting from simple cleavage of the 14,15 bond (m/z 125) is present, suggesting that the diene is in some way involved with the proton transfer.

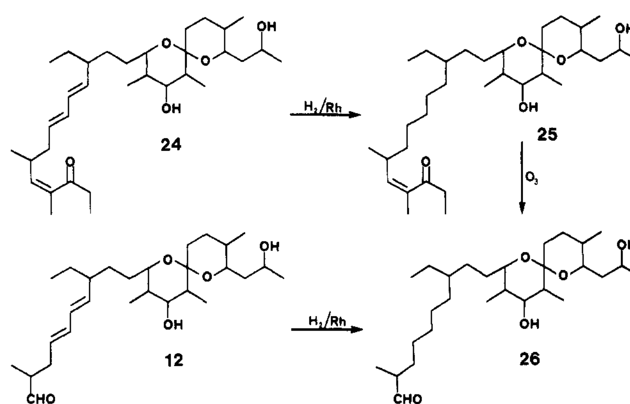
Scheme I



Scheme II



Scheme III



by a molecular ion of m/z 536 in its EIMS and UV and IR absorptions at 230 nm and 1671 cm^{-1}, respectively. Ozonolysis of **25** gave **26** which proved identical by TLC, GC, and EIMS with the compound produced by homogeneous catalytic hydrogenation of **12**. The identity of these two products and the preceeding chemical and spectroscopic data establish the structure of **24**.

Compound **24** is formed by retroaldol cleavage of the 9,10 bond, protonation of the resulting enolate, and dehydration to form the 12,13 double bond. Dehydration at this position requires a proton at C-12, thus confirming the proposed loss of the tertiary alcohol function. Competing retroaldol cleavage of the 12,13 bond predominates under these conditions as the ratio of **12** to **24** is approximately 3:1. Retoaldol cleavage of the 8,9 bond, cyclization, and dehydration produce **10** as described previously. Carbon atom 9 is the only fragment of the oligomycin C structure not directly represented in products **10**, **12**, and **24**. Its substitution is readily assigned as a secondary alcohol in analogy with the other oligomycins, since it is the initiation site for the retroaldol reactions leading to the formation of **10** and **24**.

Reconstruction of the intact molecule, including closure of the lactone at 25 as indicated by the ^1H NMR data (H-25 δ 4.88, dd, J = 11.2, 5.0 Hz) yields structure **3** for oligomycin C.

The stereochemistry of oligomycins A and C was not rigorously explored in this work. In areas where stereochemical features were fairly obvious (e.g., trans relationship of the 24 and 26 methyl groups and the trans olefin geometries), oligomycins A and C were found to be identical with oligomycin B. Furthermore, degradation products common to two (e.g., **7**, **12**) or all three (i.e., **10**) of the oligomycins appear to have the same stereochemistry on the basis of ^1H NMR spectroscopy. It seems quite likely therefore that the stereochemistry of A and C is the same as was found[11] for oligomycin B.

The probable polyketide origin of the oligomycins can be represented as follows:

$$^{1}A_1P_2P_3P_4P_5P_6P_7A_8A_9B_{10}A_{11}P_{12}P_{13}A_{14}P_{15}A_{16}A_{17}$$

where ^1A represents an acetate unit at the carboxyl end of the polyketide chain. The remaining chain is composed of units derived from acetate (A), propionate (P), and butyrate (B), terminating with an acetate unit. The absence of the 26 methyl group in the rutamycins (**4**, **5**) indicates replacement of propionate with acetate as the 13th unit of the chain. In this model, all of the odd-numbered carbons of **1**–**5** would be derived from C-1 of their respective acyl precursors. Similarly, all even-numbered chain positions would be derived from C-2. Oxygen functionalities at positions 12 and 28 must, therefore, be added subsequent to the elaboration of the polyketide chain. On the basis of these premises, oligomycin C (**3**) would be the precursor of oligomycin A (**2**) which in turn

would give rise to oligomycin B (1). Analogously, rutamycin B (5) would be the precursor of rutamycin (4).

Experimental Section

Antibiotics. The oligomycin complex was obtained from large-scale fermentations (Chas. Pfizer Co.) and consisted of crystalline mixtures of A, B, and C components. Rutamycin was kindly supplied by Dr. J. W. Chamberlin of Lilly Research Laboratories. The oligomycin complex was separated into the A, B, and C components by the method of Masamune et al.[9]

Solvents. Unless otherwise noted, all solvents were reagent grade and were used without further treatment; dry solvents were obtained by standard methods: benzene was distilled from $LiAlH_4$ and stored over 4-Å molecular sieves, CH_2Cl_2 was dried by storage over 4-Å molecular sieves, pyridine was stirred with KOH for several hours and then distilled and stored over 4-Å molecular sieves, and toluene was distilled from P_2O_5.

Ozonolyses. Ozone was either generated in a Welsbach apparatus (90 V, sample flow 0.04, total flow 0.08 mL/min) or for small-scale experiments in a Supelco microozonizer.

Chromatography. For analytical TLC, 250-μm air-dried layers of silica gel G (EM Reagents) were used. Spots were visualized by spraying with 50% aqueous sulfuric acid and charring; R_f values cited refer to analytical TLC on silica gel G unless otherwise specified. Preparative TLC plates were 750-μm layers (20 × 20 cm) of a 1:1 mixture of silica gel H and silica gel PF_{254} (EM Reagents). These plates were air-dried prior to use but were not activated further; zones were visualized by brief exposure to iodine vapor or by the use of short or long wavelength UV light. Compounds were eluted from the gel with EtOAc. All solvent system proportions given are volume to volume. Gas–liquid chromatography (GC) was performed with either a Varian Model 2700 or a Packard Model 417 instrument equipped with flame ionization detectors. Helium was employed as the carrier gas in the Varian instrument; nitrogen was the carrier gas in the Packard instrument. The carrier gas flow rate through 2 mm i.d. glass columns was adjusted to 30 mL/min. Hydrogen and compressed air flow rates to the detectors were approximately 30 and 300 mL/min, respectively.

Instrumental. Infrared spectra (IR) were recorded on a Perkin-Elmer Model 567 instrument. Ultraviolet spectra (UV) were obtained on a Beckman Model 24A recording spectrophotometer. Mass spectra were determined with an AEI MS902 instrument, using electron impact ionization at 70 eV. Samples were introduced by direct probe, while maintaining the source at the minimum temperature required for sample vaporization. High resolution mass spectra were determined on an AEI MS902 instrument at the Space Sciences Laboratory, University of California at Berkeley. Both ^{13}C and ^1H NMR spectra were obtained on a Bruker HXE-90 instrument with $CDCl_3$ as the solvent. Chemical shift values are reported in ppm (δ) downfield from internal Me_4Si standard; coupling constants are in hertz. Optical rotation measurements were performed on a Perkin-Elmer Model 141 instrument.

Base Degradation of Oligomycin A. Oligomycin A (200 mg) was dissolved in MeOH (18 mL) and 1 N aqueous NaOH (2.0 mL) was added. The solution was refluxed for 2 h and then allowed to cool before being concentrated under reduced pressure to remove the majority of the MeOH. Water (5 mL) was added to the residue which was then acidified to pH 1–2 with 1 N HCl. BHT (1 mg) and added to prevent autoxidation. The mixture was extracted with $CHCl_3$ (3 × 50 mL). The volume of the organic phase was reduced to 25 mL by evaporation under reduced pressure and the acid products were extracted with 5% $NaHCO_3$ (3 × 20 mL). The $CHCl_3$ layer, which contained neutral products, was dried over $MgSO_4$ and reserved.

Isolation of Compound 12 from Neutral Fraction. The neutral fraction was evaporated to dryness, taken up in a small volume of EtOAc, and chromatographed on three preparative TLC plates developed in hexane/EtOAc (5/3). The zone centered at R_f 0.2 yielded 12 (28 mg) as a glass with a TLC R_f 0.29 in hexane/EtOAc (5/3): IR (CCl_4) ν_{max} 3607, 3470, 2970, 2932, 2880, 2720, 1721, 1458, 1386, 1019, 990, 981 cm^{-1}; UV (CH_3OH) λ_{max} 226 (ε 3.9 × 10^4), 230 (ε 3.9 × 10^4), 239 (ε 2.6 × 10^4); EIMS, m/z (relative intensity) 464 (M$^+$, 4), 446 (16), 389 (8.5), 244 (7.5), 223 (9.5), 213 (32), 185 (100), 184 (62), 167 (48), 155 (18), 149 (22), 137 (20), 95 (80); $[\alpha]^{25}_D$ −47.7° (c 0.524, CH_3OH); ^1H NMR δ 9.65 (1 H, d, 1.5 Hz), 6.10 (1 H, dd, 14.6, 10.5 Hz), 5.95 (1 H, m), 5.45 (2 H, m), 3.77 (1 H, dd, 10.5, 5.0 Hz), 1.23 (3 H, d, 5.9 Hz), 1.11 (3 H, d, 6.4 Hz), 1.05 (3 H, d, 6.4 Hz), 0.916 (3 H, d, 6.4 Hz), 0.843 (3 H, d, 6.8 Hz); ^{13}C NMR δ 204.7, 137.9, 133.2, 129.8, 127.4, 99.3, 73.0, 70.3, 67.5, 65.3, 46.3, 44.7, 41.9, 40.4, 38.5, 33.7, 31.6, 30.7, 30.4, 27.8, 26.6, 26.0, 24.1, 13.2, 11.8 (2 unresolved signals), 11.4, 4.9.

Isolation of Compounds 8 and 11 from the Acid Fraction. The bicarbonate layer was acidified to pH 1–2 with 6 N HCl added dropwise and then the solution was extracted with ether (3 × 50 mL), and this extract was treated with ethereal diazomethane until the yellow color persisted. After 10 min, excess diazomethane was removed with a stream of nitrogen, and the solution was then dried over $MgSO_4$. The methylated fraction was chromatographed by preparative TLC developed with benzene/EtOAc (5/3). The zone between R_f 0.06–0.12 contained 8: TLC R_f 0.37 in 5% MeOH in $CHCl_3$; IR ($CHCl_3$) ν_{max} 3510; 2968, 2921, 2873, 1727, 1715, 1455, 1380, 1353, 1170, 1012, 998 cm^{-1}; EIMS, m/z (relative intensity) 297 (M − H_2O − CH_3CO, 62), 217 (26), 211 (100), 199 (29), 187 (56), 155 (19), 137 (81), 125 (19), 124 (28), 115 (20); ^1H NMR δ 4.54 (1 H, d, 2.9 Hz), 3.70 (3 H, s), 3.47 (2 H, bs), 2.92 (1 H, dd, 15.4, 2.9 Hz), 2.46 (1 H, dd, 15.4, 5.1 Hz), 2.31 (3 H, s), 1.13 (3 H, d, 6.4 Hz), 1.05 (3 H, d, 6.6 Hz), 1.01 (3 H, d, 6.10 Hz), 0.851 (3 H, d, 6.8 Hz); $[\alpha]^{25}_D$ −73° (c 0.10, CH_3OH).

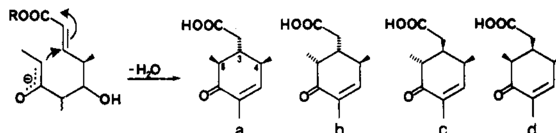
The zone R_f 0.50–0.63 contained the product mixture[25] designated 11 which appeared as a diffuse, elongated spot at TLC R_f 0.60 in 1% MeOH in $CHCl_3$: IR (CCl_4) ν_{max} 2979, 2930, 2880, 1739, 1676, 1433, 1372, 1239, 1170, 1046 cm^{-1}; UV (CH_3OH) λ_{max} 235 nm; EIMS m/z (relative intensity) 210 (M$^+$, 16), 179 (11), 138 (11), 137 (100), 136 (95), 135 (28), 122 (21), 121 (13), 109 (14), 96 (36); ^1H NMR δ 6.43 (1 H, bs), 3.68 (3 H, s), 2.35 (2 H, bs), 1.72 (3 H, bs); $[\alpha]^{25}_D$ −56° (c 0.106, CH_3OH).

$NaBH_4$ Reduction of 12. Compound 13. Approximately 30 mg of 12 was treated with an excess of $NaBH_4$ in 1 mL of EtOH (100%) for 20 min at room temperature with stirring. Water (5 mL) and 1 N NaOH (5 mL) were added, and the mixture was extracted with ether (3 × 20 mL). The ether layer was washed with saturated NaCl and dried over Na_2SO_4 to obtain 28 mg of

(25) It was recognized from analysis of the ^1H NMR data that 11 was actually a mixture of isomers. These were separable by capillary GC as follows: GC column, glass, 400 ft × 0.75 mm i.d.; liquid phase, SE 30; temperatures, column oven 170 °C, injector 200 °C, detector 200 °C; flow rates, He (carrier) 7 mL/min, H_2 30 mL/min, air 300 mL/min. The product showed three components in this analysis.

t_R (min)	17.5	18.2	18.5
peak height (mm)	89	43	51

Consideration of the mechanism of formation of 10 by base degradation permits the following explanation for the origin of the isomeric products. The reaction scheme shown below depicts the Michael-type cyclization of an intermediate enolate, plus dehydration to form four possible products a, b, c, and d, which differ in the relative configuration of the C-3 and C-8 substituents. The relative configurations adopted by car-



bons 3 and 8 are influenced by the configuration at C-4, which is expected to be retained under the reaction conditions. Since Michael condensations are reversible, one would expect the thermodynamically most stable products to predominate in the mixture. Product a should be the most stable because the substituents at C-3, 4, and 8 are able to assume an all equatorial (or pseudoequatorial) conformational with neighboring substituents in a trans relationship. Products b and c should rank next in stability; their energetically most favorable conformation is that in which the C-3 acetic acid substituent is equatorial. In this conformation both products have one cis equatorial acetic acid–pseudoaxial methyl relationship and one 1,3-pseudoaxial methyl–pesudoaxial hydrogen relationship, which make them less favored than a. Similar arguments apply to all-cis d which is expected to be the least stable product. GC showed three components in a ratio of 2.1:1.0:1.2. Although these isomers have not been individually characterized, it is reasonable to postulate that the major component is a and the other two are b and c and that no d was formed.

Table III. Characteristic EIMS Fragment Ions of Selected Degradation Products

	a	b	c	d	e	f	g
12	337.23922[a] $C_{20}H_{33}O_4$ (-4.0)	334.25078 $C_{21}H_{34}O_3$ (0.1)		224.17724 $C_{14}H_{24}O_2$ (1.7)	155.10502[b] $C_9H_{15}O_2$ (14.1)	184.14498 $C_{11}H_{20}O_2$ (7.3)	213.14841 $C_{12}H_{21}O_3$ (3.1)
13				224 (14)[c]	155 (17)[b]	184 (100)	213 (41)
16			490 (7)	406 (7)	355 (14)	366 (39)	
17	253 (24)[b]	268 (11)	310 (24)		173 (58)	184 (100)	213 (20)
18	245 (30)	242 (15)	284 (55)	224 (11)	173 (71)	184 (100)	213 (11)
19	329 (10)		268 (25)	266 (39)	215 (39)	226 (100)	
24	405.29385 $C_{25}H_{41}O_4$ (16.4)	402.31605 $C_{26}H_{42}O_3$ (-6.6)		224.17836 $C_{14}H_{24}O_2$ (-3.2)	173.11908 $C_9H_{17}O_3$ (-7.6)	184.14652 $C_{11}H_{20}O_2$ (-1.0)	213.15133 $C_{12}H_{21}O_3$ (-10.6)
25	409 (48)	388 (23)[b]	448 (22)		173 (30)	184 (100)	
26	341 (28)		380 (20)		173 (60)	184 (100)	

[a] High resolution data includes measured m/z value, derived elemental composition, and (deviation from theoretical value in ppm). [b] m/z value for indicated ion − H_2O. [c] Low resolution data: m/z (relative intensity).

triol **13** as a glass. This product had a TLC R_f value of 0.11 as opposed to 0.26 for **12** in hexane/EtOAc (5/3). **13**: EIMS, m/z (relative intensity) 466 (M^+, 2.5), 388 (15), 246 (8), 225 (10), 224 (14), 213 (41), 185 (92), 184 (100), 167 (49), 155 (17), 149 (21), 137 (16).

***p*-Bromobenzoylation of 13. Compound 14.** Triol **13** (28 mg) was dissolved in dry pyridine (1 mL) and *p*-bromobenzoyl chloride (85 mg) was added. The solution was allowed to stand in a dessicator 36 h before workup. The mixture was then poured onto ice in a separatory funnel and extracted with a total of 50 mL of $CHCl_3$. The $CHCl_3$ layer was washed with 1 N HCl (200 mL) followed by 5% $NaHCO_3$. Upon evaporation unextracted *p*-bromobenzoic acid was found; this was successfully removed by trituration of the product with hexane and filtering to remove the undissolved acid. Compound **14** was purified by preparative TLC developed with 15% EtOAc/hexane. The zone R_f 0.55–0.70 contained **14** which had a TLC R_f value of 0.42 in 10% EtOAc/hexane: IR (CCl_4) ν_{max} 2970, 2940, 1712, 1591, 1488, 1460, 1402, 1275, 1178, 1118, 1108, 1072, 1016, 990, 967, 852 cm^{-1}; ^1H NMR δ 7.64 (12 H, AA'XX'), 5.99 (1 H, dd, 14, 10 Hz), 5.28 (1 H, dd, 11.2, 4.9 Hz), 4.16 (2 H, bd, 5.1 Hz), 3.84 (1 H, bm), 3.59 (1 H, bm), 1.38 (3 H, d, 6.1 Hz), 0.922 (3 H, d, 5.9 Hz).

Ozonolysis of 14. Compounds 15 and 16. Compound **14** (11.3 mg) was dissolved in CH_2Cl_2 (0.5 mL) containing 5 μL of dry pyridine. Ozone was bubbled through to the starch/KI endpoint at −78 °C and then excess ozone was removed with a stream of nitrogen. The solvents were evaporated and the residue was chromatographed by preparative TLC developed with 15% EtOAc/hexane. The zone R_f 0.10–0.15 yielded **15**: R_f 0.25 in 10% EtOAc/hexane; IR (CCl_4) ν_{max} 2955, 2810, 1723, 1590, 1480, 1396, 1269, 1172, 1112, 1100, 1070, 1014, 848, 680 cm^{-1}; ^1H NMR δ 9.80 (1 H, dd, 1.7, 14.7 Hz), 7.72 (4 H, AA'XX'), 4.2 (2 H, AB), 2.5 (2 H, AB), 1.11 (3 H, d, 6.6 Hz); EIMS, m/z (relative intensity) 286 (M^+, 1.6), 284 (M^+, 1.5), 258 (9), 256 (9), 203 (38), 202 (35), 201 (37), 200 (34), 185 (99), 183 (100), 157 (40), 155 (38), 123 (30), 105 (90), 101 (20), 56 (88), 44 (36); $[\alpha]^{25}_D$ −6.5° (*c* 0.2, CH_3OH).

The zone R_f 0.15–0.27 gave compound **16**: TLC R_f 0.39 in 10% EtOAc/hexane; ^1H NMR δ 9.38 (1 H, d, 7.1 Hz), 7.80 (8 H, AA'XX'), 6.24 (1 H, dd, 7.9, 15.5 Hz), 5.91 (1 H, dd, 7.3, 15.5 Hz), 5.30 (2 H, m), 3.81 (1 H, m) 3.62 (1 H, m), 1.39 (3 H, d, 6.1 Hz), 0.987 (3 H, d, 6.6 Hz), 0.956 (3 H, d, 6.6 Hz), 0.835 (3 H, d, 6.8 Hz), 0.745 (3 H, t, 7.3 Hz); EIMS, m/z (relative intensity) 764 (M^+, 1), 564 (7), 563 (6), 492 (7), 490 (7), 408 (7), 406 (7), 368 (39), 366 (39), 357 (14), 355 (14), 312 (11), 310 (11), 248 (13), 206 (20), 185 (97), 183 (100).

Ozonolysis of 17. Compounds 18 and 19. Compound **16** (0.5 mg) was reduced in the manner described for **12** by using an excess (1 mg) of $NaBH_4$. The product was then stirred with excess NaOH in 95% EtOH for 3 h, diluted with saturated NaCl, and extracted with ether. The extract was washed with 5% $NaHCO_3$ and dried over $MgSO_4$ and subjected to preparative TLC with 10% $MeOH/CHCl_3$. The zone R_f 0.35–0.47 yielded **17**, R_f 0.275 in 7% $MeOH/CHCl_3$: EIMS, m/z (relative intensity) 398 (M^+, 3), 380 (6), 310 (24), 268 (11), 253 (24), 235 (9), 213 (20), 185 (22), 184 (100), 179 (20), 173 (58), 155 (53), 138 (21), 137 (19). Approximately 50 μg of **17** was dissolved in CH_2Cl_2 (0.1 mL) containing pyridine (5 μL). The olefin was ozonized to the starch–KI endpoint at −78 °C. Excess ozone was removed with a stream of nitrogen and the solvents were removed by evaporation. Absolute EtOH (0.1 mL) and $NaBH_4$ (1 mg) were added and the mixture was stirred for 10 min at room temperature. Water (0.2 mL) was added and the product **18** extracted into ether. EIMS, m/z (relative intensity) 372 (M^+, 5), 354 (4), 284 (55), 245 (30), 242 (15), 227 (17), 224 (11), 213 (11), 209 (18), 185 (23), 184 (100), 173 (71), 155 (78).

The triol **18** was treated with Ac_2O in pyridine (1:3) to obtain **19**: TLC R_f 0.39 in 1% $MeOH/CHCl_3$, GC retention time of 16.05 min under the following conditions: column, glass, 6 ft × 2 mm i.d.; liquid phase, 3% OV-101 on Varaport 30, 100–120 mesh; temperatures, initial 200 °C for 5 min, final 245 °C, rate 4°/min; injector 300 °C; detector 300 °C. EIMS, m/z (relative intensity) 498 (M^+, 4), 438 (8), 368 (25), 329 (9.5), 267 (14), 266 (39), 226 (100), 215 (39), 164 (22), 155 (47), 149 (22), 137 (21), 123 (53).

Ozonolysis of Oligomycin B. Compound 20. Oligomycin B (281 mg) was dissolved in EtOAc (4 mL) at −78 °C. Ozone was bubbled through for 5 min. Excess ozone was then removed with a stream of nitrogen. Adams catalyst (PtO_2, 25 mg) was added and the ozonides were reduced at 50 psig H_2 for 36 h at room temperature. After filtration to remove the catalyst the resulting glycolate ester was hydrolyzed by using excess base (NaOH) in MeOH. The solution was acidified with 1 N HCl and the product extracted into ether. The ether layer was washed with water and saturated NaCl and dried over $MgSO_4$. Preparative TLC of the hydrolysis product developed in 7% $MeOH/CHCl_3$ yielded 40 mg of **20**: R_f 0.36 in 9% $MeOH/CHCl_3$; IR ($CHCl_3$) ν_{max} 3380, 2970, 2880, 1729, 1462, 1388, 999, 760 cm^{-1}; EIMS, m/z (relative intensity) 386 (M^+, 0.5), 303 (5), 246 (17), 245 (100), 227 (42), 209 (32), 191 (8), 181 (12), 171 (23), 157 (18), 153 (21), 139 (20), 135 (23), 99 (74); ^1H NMR δ 4.57 (1 H, bd, 10.2 Hz), 3.75 (1 H, dd, 10.5, 5.5 Hz), 3.50 (2 H, m), 3.01 (1 H, dd, 14.9, 5.9 Hz), 1.24 (3 H, d, 6.8 Hz); ^{13}C NMR δ 202.4, 100.4, 73.1, 72.6, 67.1, 64.7, 44.1, 41.9, 38.2, 37.2, 34.0, 28.9, 26.8, 24.9, 23.4, 12.8, 11.7, 11.4, 4.7; $[\alpha]^{25}_D$ −107° (*c* 0.658, CH_3OH).

Conversion of 20 to 23. Compound **20** (0.5 mg) was acetylated in 1:3 Ac_2O/pyridine at 50 °C for 5 h. The solution was evaporated to dryness with a stream of nitrogen. Toluenesulfonyl hydrazide (1 mg) and 0.1 N methanolic HCl (35 μL) were added, and the mixture was let stand at 30 °C overnight and then heated at 62 °C for 2 h. Preparative TLC of the crude reaction mixture in 1% $MeOH/CHCl_3$ yielded a broad zone at R_f 0.32 which was thought to contain pure **22** (R_f 0.18, 1% $MeOH/CHCl_3$). This material was dissolved in dry toluene (0.3 mL) to which LiH (3 mg) was added. The suspension was heated at 114 °C in a sealed vial for 5.5 h and filtered, and the product was subjected to preparative TLC developed with 2% $MeOH/CHCl_3$. The zone R_f 0.54–0.66 by EIMS contained **23** as the major product plus some unreacted **21**. Since the separation of these two compounds was expected to be difficult, the mixture was redissolved in 0.1 N methanolic HCl (35 μL), *p*-toluenesulfonyl hydrazide (1 mg) was added, and the solution was heated at 65 °C for 6.25 h. All volatiles were then evaporated under a stream of nitrogen. Dry toluene (50 mL) and LiH (3 mg) were added and the suspension was heated in a sealed vial at 115 °C for 12.5 h. EIMS of the crude product showed only those ions attributable to **23** and **19**.[26] This

product was subjected to preparative TLC developed with 1% $MeOH/CHCl_3$. A broad zone R_f 0.20–0.40 contained product **23**.

EIMS for **21**: m/z (relative intensity) 512 (M^+, 0.4), 494 (1), 484 (4), 329 (4), 269 (2), 209 (6), 191 (3), 177 (4), 157 (16), 156 (100), 149 (24), 141 (9), 114 (13). EIMS for **23**: m/z (relative intensity) 498 (2), 496 (M^+, 2), 438 (6), 436 (7), 368 (11), 366 (5), 329 (8), 305 (17), 266 (16), 264 (7), 226 (33), 224 (100), 215 (14), 213 (42), 154 (36), 143 (30).

Catalytic Reduction of 23 to 19. Product **23** from the above experiment was dissolved in EtOAc (1.5 mL) to which was added PtO_2 (5 mg). The reduction was carried out under H_2 (40 psig) for 10 h at room temperature and then filtered, and the solvent was removed under reduced pressure. Product **19** was purified by chromatography on a silica gel column (1 g, Biorad Bio Sil A) eluted with 5 mL of hexane to remove the bulk of the impurities and an additional 5 mL to elute compound **19**. This compound was identical by TLC, GC, and EIMS with the compound derived from **12**.

Base Degradation of Oligomycin C. Oligomycin C (50 mg) was dissolved in MeOH (6 mL) plus 0.1 N NaOH (3 mL). The solution was refluxed for 2.5 h. Water (5 mL) and BHT (1 mg) were added and the bulk of the MeOH was removed under reduced pressure. The resulting cloudy solution was extracted with $CHCl_3$ (50 mL). The $CHCl_3$ layer containing neutral products was reserved. The aqueous layer was acidified with 1 N HCl (2.5 mL) and extracted with ether (2 × 50 mL). The ether layer containing acidic products was washed with saturated NaCl and dried over Na_2SO_4.

Isolation of 11. The ether extract was treated with ethereal diazomethane and then chromatographed by preparative TLC in hexane/EtOAc (5/3). Compound **11** (1.5 mg) was found in the zone of R_f 0.46–0.55.

Isolation of Compounds 12 and 24. The neutral fraction was chromatographed on a silica gel column (20 g, 1 × 58 cm). The mobile phase was hexane/EtOAc (5/3), flowing at a rate of 1 mL/min; 3.2-mL fractions were collected. Fractions 15–19 were combined and chromatographed by preparative TLC in hexane/EtOAc (5/3). The zone of R_f 0.31–0.39 was eluted and combined with fraction 20 to give pure **24** (2 mg), R_f 0.35 (hexane/EtOAc, 5/3); IR (CCl_4) ν_{max} 3630, 3500, 2963, 2935, 2880, 1671, 1458, 1387, 1223, 1021, 987 cm^{-1}; UV (CH_3OH) λ_{max} 225 (ϵ 5.6 × 10^4), 230 (ϵ 5.5 × 10^4), 242 (ϵ 3.5 × 10^4); EIMS, m/z (relative intensity) 532 (M^+, 1), 514 (4), 407 (5), 389 (20), 225 (8), 213 (12), 185 (42), 184 (22), 167 (16), 126 (100), 125 (19); ^1H NMR δ 6.36 (1 H, d, 9.5 Hz), 5.95 (2 H, m), 5.35 (2 H, m), 3.72 (1 H, dd, 10.7, 4.9 Hz), 2.66 (2 H, q, 7.3 Hz), 1.77 (3 H, d, 1.0 Hz), 1.21 (3 H, d, 6.4 Hz), 1.04 (3 H, d, 6.8 Hz), 0.908 (3 H, d, 7.3 Hz), 0.830 (3 H, d, 6.8 Hz); $[\alpha]^{25}_D$ −39.3° (c 0.29, CH_3OH).

Fractions 23–29 were combined to give **12** (9.1 mg).

Selective Hydrogenation of 24 to 25. Compound **24** (2 mg) was dissolved in dry benzene (1.5 mL) and tris(triphenylphosphine)rhodium(I) chloride (5 mg) was added. The solution was shaken in a Parr pressure apparatus at 10 psig H_2 for 18 h at room temperature. Preparative TLC of the reaction mixture with hexane/EtOAc (5/3) yielded compound **25** (0.5 mg) in a zone centered at R_f 0.40. Analytical TLC in hexane/EtOAc (5/3) gave an R_f value of 0.42: IR (CCl_4) ν_{max} 2967, 2930, 1671, 1540, 1460, 1380, 1223, 980 cm^{-1}; UV (CH_3OH) λ_{max} 226 nm; EIMS, m/z, (relative intensity) 536 (M^+, 7), 518 (8), 448 (22), 409 (48), 388 (22), 350 (13), 307 (19), 295 (16), 185 (24), 184 (100), 173 (30), 155 (30), 137 (20), 125 (18).

Ozonolysis of 25 to 26. Compound **25** (0.3 mg) was dissolved in dry CH_2Cl_2 (0.25 mL) containing dry pyridine (5 μL). Ozone was bubbled through the solution at −78 °C to the starch–KI endpoint. Solvents were evaporated with a stream of nitrogen to give **26**: R_f 0.25 in hexane/EtOAc (2/1); EIMS, m/z (relative intensity) 468 (M^+, 2), 450 (4), 388 (20), 341 (28), 323 (16), 310 (28), 267 (12), 185 (20), 184 (100), 173 (60), 155 (54), 149 (20), 143 (16), 137 (28).

Selective Hydrogenation of 12 to 26. Compound **12** (1.5 mg) and tris(triphenylphosphine)rhodium(I) chloride (5 mg) were dissolved in benzene (1.5 mL). The solution was shaken in a Parr apparatus at 10 psig H_2 for 14 h. The reaction mixture was subjected to preparative TLC in hexane/EtOAc (3/2). The zone centered at R_f 0.41 contained both the tetra- and dihydro products by EIMS. The material from this zone was dissolved in CH_2Cl_2 (0.2 mL) containing pyridine (5 μL) and ozonized to the starch–KI endpoint. The solvents were evaporated with a stream of nitrogen and the residue separated by preparative TLC in hexane/EtOAc (4/3). The zone at R_f 0.43 was now free of dihydro products and contained pure **26**: EIMS, m/z (relative intensity) 468 (M^+, 2), 450 (2), 380 (21), 341 (28), 323 (13), 310 (30), 267 (10), 185 (20), 184 (100), 173 (73), 155 (63), 143 (18), 137 (20).

Chromatographic Comparison of Compound 26 Produced from 24 with Compound 26 Derived from 12. The two compounds co-chromatograph with an R_f value of 0.25 in hexane/EtOAc (2/1) and on GLC with a retention time of 7.00 min under the conditions listed: GC column, glass, 6 ft × 2 mm i.d.; liquid phase, 3% OV-101 on Varaport 30, 100–120 mesh; temperatures; column oven initial 225 °C, final 295 °C; rate 10°/min; injector 325 °C; detector 325 °C.

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(26) Evidently sufficient H_2 was generated in this reaction to cause partial hydrogenation of the olefin of 23.