Structure and Absolute Stereochemistry of 21-Hydroxyoligomycin A⊥

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21-Hydroxyoligomycin A (**1**) was isolated from *Streptomyces cyaneogriseus* ssp. *noncyanogenus* (LL-F28249) and fully characterized by NMR and single-crystal X-ray diffraction methods. The complete ^{1}H and ^{13}C NMR chemical shift assignments for **1** were made using 2D NMR experiments, and the chirality at C-21 was deduced to be *R* from a *J*-based configuration analysis. The absolute configuration at C-21 and at the other 18 chiral centers in the molecule were independently confirmed by anomalous dispersion measurements on a crystal of the chloroform methanol solvate of 21-hydroxyoligomycin A (**1**).

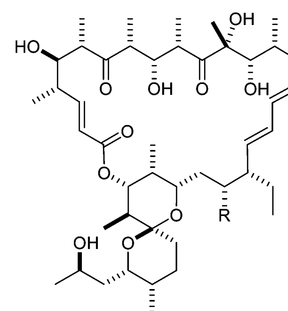
First isolated from *Streptomyces diastatochromogenes* in 1954, oligomycins are neutral macrolides characterized by a 26-membered ring fused to a bicyclic spiroketal.[1] Members of the oligomycin family display a wide variety of bioactivity including anticancer, antifungal, nematocidal, insecticidal, and immunosuppressive effects.[2−5] While most oligomycin-type compounds are inactive against bacteria, oligomycin E is reported to be active against Gram-positive bacteria but inactive against Gram-negative bacteria.[6] Furthermore, their use as inhibitors of mitochondrial ATPase has aided in the biochemical understanding of the mechanism of oxidative phosphorylation.[7] Most recently, oligomycin was reported to block Pgp efflux activity, thereby increasing the potency of some antitumor agents.[8]

In addition to the oligomycin group, other examples of the spiroketal-containing class of macrolides include cytovaricin, rutamycins A and B, A82548A, phthoramycin, kaimonolide, and the dunaimycins.[9−14] The absolute configuration of both cytovaricin and A82548A was determined by hydrolysis and characterization of the resulting sugar moieties.[11,15] Degradation and asymmetric synthesis of known degradation products incorporating the spiroketal unit were used to establish the absolute stereochemistry of both rutamycin and oligomycin B.[16,17] In addition, total synthesis of cytovaricin, rutamycin, and oligomycin C further substantiated the assigned absolute stereochemistry.[18,19]

This report describes the complete structure elucidation of 21-hydroxyoligomycin A (**1**) including comprehensive ^{1}H and ^{13}C NMR chemical shift assignments. The *J*-based configuration method utilizing several NMR experiments including G-BIRD$_{R,X}$-HSQMBC, HETLOC, D-HMBC, and E.COSY was used to establish the stereochemistry at C-21 relative to the spiroketal unit.[20] The NMR-derived structure was ultimately confirmed by a single-crystal X-ray crystallographic analysis of the chloroform methanol solvate of 21-hydroxyoligomycin A.

Results and Discussion

LL-F28249ω (**1**) was isolated from *Streptomyces cyaneogriseus* ssp. *noncyanogenus* (LL-F28249) in the course of a program focused on the discovery of novel antiparasitic agents. The planar structure was determined by detailed analysis of NMR data in



1 21-hydroxyoligomycin A R = OH
2 oligomycin A R=H

conjunction with HRMS and IR data. The molecular formula for LL-F28249ω (**1**) was determined to be $C_{45}H_{74}O_{12}$ by HRMS (*m/z* 807.5274 $[M + H]^+$). Initial inspection of both the ^{1}H and ^{13}C NMR spectra indicated LL-F28249ω (**1**) to be a member of the oligomycin class of compounds.[21] Comparison of the ^{13}C NMR data of LL-F28249ω (**1**) with those of oligomycin A in deuteriochloroform ($CDCl_3$) revealed significant similarities (see Table 1). On the basis of the molecular formula, LL-F28249ω (**1**) differs from oligomycin A by the addition of a single oxygen atom. The presence of an additional oxygenated carbon is evident in the ^{13}C NMR spectrum of LL-F28249ω (**1**); seven methine signals exist between 60 and 80 ppm in the ^{13}C NMR spectrum of oligomycin A, whereas eight methine signals appear in the same region in the DEPT spectrum of **1**. Furthermore, LL-F28249ω (**1**) has one less methylene carbon signal in the region from 20 to 40 ppm as compared to oligomycin A. More detailed analysis of the ^{13}C NMR spectra showed a significant upfield shift of C-19 from δ 137.6 to δ 133.8, a downfield shift of C-20 from δ 45.9 to δ 52.1, a downfield shift of C-23 from δ 68.8 to δ 70.2, and an upfield shift of C-41 from δ 28.2 to δ 24.2 when comparing the NMR spectrum of oligomycin A to that of LL-F28249ω (**1**). These differences suggest that the additional oxygen atom in LL-F28249ω (**1**) is located on either C-21 or C-22.

Using 2D NMR experiments including H,H-COSY, HSQC, and HMBC, the placement of the hydroxyl group on C-21 was definitively determined by the key correlations described below and shown in Figure 1. The alkenyl proton H-19 (δ 5.51 ppm) is correlated to H-20 (δ 1.83 ppm), which in turn is correlated to a proton at δ 4.04 ppm, as is evident in the COSY spectrum. From analysis of the HSQC spectrum, the proton at δ 4.04 ppm is attached to a carbon with a chemical shift of δ 73.6 ppm. Furthermore, the alkenyl protons of both C-18 and C-19 (δ 5.97 and 5.51 ppm, respectively) are correlated to the oxygenated methine at δ 73.6 ppm, as seen in the HMBC spectrum. In addition, the methylene

⊥ Dedicated to the late Dr. Kenneth L. Rinehart of the University of Illinois at Urbana−Champaign for his pioneering work on bioactive natural products.

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Table 1. NMR Spectroscopic Data for 21-Hydroxyoligomycin A (**1**) and Oligomycin A (**2**) in $CDCl_3$

	21-hydroxyoligomycin A (**1**)		oligomycin A (**2**)	
position	δ_C, mult.	δ_H (J in Hz)	δ_C	δ_H
1	165.2, qC		165.2	
2	122.9, CH	5.83, d (15.7)	122.6	5.82
3	148.7, CH	6.65, dd (10.2, 15.6)	148.2	6.65
4	40.5, CH	2.41, ddq (6.5, 10.1, 10.1)	39.8	2.38
5	73.1, CH	3.81, dd (0.9, 10.1)	72.8	3.78
6	46.8, CH	2.71, dq (1.0, 7.3)	46.1	2.72
7	220.5, qC[a]		220.4	
8	45.8, CH	2.77, dq (2.7, 7.0)	46.3	2.78
9	72.8, CH	3.97, dd (2.7, 8.9)	72.3	3.96
10	42.2, CH	3.66, dq (6.8, 9.0)	41.8	3.61
11	220.4, qC[a]		219.8	
12	83.3, qC		82.6	
13	72.4, CH	3.97, d (1.8)	71.9	3.92
14	33.8, CH	1.92, m[b]	33.3	1.89
15	38.7, CH_2	2.21, m[b]	38.3	1.98
		2.01, m[b]		2.18
16	130.1, CH	5.49, ddd (3.9, 11.0, 15.0)	129.2	5.45
17	132.6, CH	6.09, ddd (1.4, 10.4, 14.9)	132.2	6.03
18	132.1, CH	5.97, dd (10.4, 15.2)	130.1	5.93
19	133.8, CH	5.51, dd (10.2, 15.0)	137.6	5.24
20	52.1, CH	1.83, m[b]	45.9	1.84
21	73.6, CH	4.04, m[b]	31.2	1.53
				1.40
22	37.7, CH_2	1.76, dt (10.4, 10.4, 15.3)	30.8	1.62
		1.28, m[b]		1.04
23	70.2, CH	4.18, ddd (1.9, 3.4, 10.0)	68.8	3.81
24	35.6, CH	2.2, m[b]	35.7	2.12
25	75.9, CH	4.96, dd (4.9, 11.4)	76.0	4.97
26	37.8, CH	1.87, dq (6.6, 11.4)	37.4	1.81
27	100.4, qC		99.1	
28	25.8, CH_2	1.95, m[b]	25.7	1.92
		1.26, m[b]		1.24
29	27.1, CH_2	1.95, m[b]	26.3	2.11
		1.45, m[b]		1.42
30	30.4, CH	1.62, m[b]	30.3	1.57
31	67.5, CH	4.06, m[b]	67.0	3.98
32	42.9, CH_2	1.65, m[b]	42.3	1.60
		1.25, m[b]		1.28
33	64.5, CH	4.06, m[b]	64.3	4.03
34	25.1, CH_3	1.25, d (6.2)	24.5	1.23
35	18.3, CH_3	1.2, d (6.5)	17.8	1.19
36	8.5, CH_3	1.08, d (7.3)	8.2	1.07
37	9.2, CH_3	1.06, d (7.0)	9.3	1.04
38	14.7, CH_3	1.15, d (6.9)	13.9	1.11
39	21.2, CH_3	1.14, s	20.8	1.13
40	14.8, CH_3	1.03, d (6.7)	14.0	1.00
41	24.2, CH_2	1.59, m[b]	28.2	1.38
		1.51, m[b]		1.29
42	12.5, CH_3	0.87, t (7.4)	11.9	0.82
43	6.3, CH_3	0.89, d (7.1)	5.9	0.84
44	12, CH_3	1.01, d (6.6)	11.6	0.98
45	11.4, CH_3	0.92, d (7.0)	11.1	0.91

[a] Assignments may be interchanged. [b] Signal partially obscured.

protons of C-41 (δ 1.51 and 1.59 ppm) show a three-bond HMBC correlation to the carbon at δ 73.6 ppm.

Lending support for the assignment of C-22 as a methylene group, H-23 (δ 4.18 ppm) shows COSY correlations to two protons at δ 1.28 and 1.78 ppm. These two protons are located on a carbon at δ 37.9 ppm as determined from analysis of the HSQC spectrum. The assignment of C-22 as a methylene group is further substantiated by the three-bond HMBC correlation of the methylene protons δ 1.28 and 1.76 ppm to C-24 (δ 35.6 ppm).

Furthermore, assignment of C-21 in LL-F28249ω (**1**) as a hydroxylated methine is in good agreement with the reported polyketide biosynthetic origin of the oligomycin family; C-21 originates from C-1 of acetate as previously demonstrated by the incorporation of labeled precursors.[22]

Thus LL-F28249ω (**1**) is trivially named 21-hydroxyoligomycin A. The complete ^{1}H and ^{13}C chemical shift assignments for LL-F28249ω (**1**) were determined using the above-mentioned combination of 2D NMR experiments and are reported in Table 1 along with the assignments for oligomycin A. The reported assignments for oligomycin A are in good agreement with published data with a few exceptions. In one case, the reported assignments of C-36 and C-37 should be interchanged.[2] In the other example, the shifts reported for C-8 and C-10 should be interchanged in addition to those for C-37 and C-38.[5]

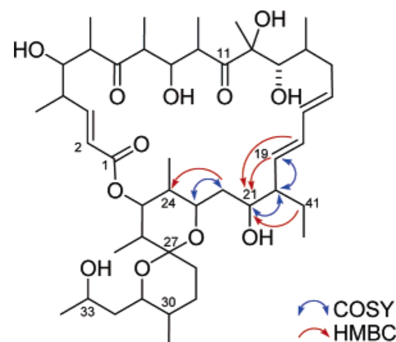

Figure 1. Select COSY and HMBC correlations for 21-hydroxyoligomycin A.

The relative stereochemistry of 21-hydroxyoligomycin A (**1**) was established using the J-based configuration analysis method in conjunction with analyses of observed NOE correlations. On the basis of the similarity of the NMR spectrum of 21-hydroxyoligomycin A (**1**) with that of oligomycin A and the growing literature evidence for a common absolute configurational assignment within the family of spiroketal macrolides, the configuration of like stereocenters was postulated to be the same for 21-hydroxyoligomycin A (**1**) as for oligomycin A. In the oligomycin family, the configuration at C-23 has been assigned as S* and that at C-20 as R*. Because the proton NMR signals for both H-20 and H-21 are overlapping multiplets, the $^3J_{HH}$ coupling constant could not be directly measured and thus relating the stereochemistry of C-20 with that of C-21 was not pursued. Therefore the relative configuration at C-21 was determined by relating H-21 to H-23 through the diastereotopic methylene protons on C-22.

The determination of the relative stereochemistry of C-21−C-22 was accomplished by analysis of all six possible rotamers and comparison of the theoretical and experimental homonuclear and long-range heteronuclear coupling constants in conjunction with observed NOEs (see Figure 2). While the multiplicity of the NMR signals for both H-21 and H-22a could not be measured because of overlap with other signals, the NMR signal for H-22b is an isolated dt with J = (10.4), 10.4, 15.3 Hz at δ 1.76 ppm. On the basis of these values, H-22b and H-21 are in a *trans* orientation to each other, as are H-22b and H-23.

The $^3J_{H21H22b}$ of 10.4 Hz indicated that H-21 and H-22b are in an *anti* relationship. Furthermore, it can be inferred that H-21 and H-22a must be in a *gauche* relationship, although the homonuclear coupling constant $^3J_{H21H22a}$ is not directly measurable from the ^{1}H NMR spectrum. As a result of this homonuclear coupling constant, four of the six possible conformations can be excluded from further considerations (rotamers C and F remaining, Figure 2). Rotamers C and F (Figure 2) can be differentiated by the magnitude of the $^3J_{C20Ha}$ and the $^2J_{C21Ha}$ couplings as well as dipolar couplings. The observed long-range heteronuclear coupling constants ($^3J_{C20Ha}$ = 2.5 Hz and $^2J_{C21Ha}$ = 2.0 Hz) showed rotamer C to be the predominant configuration. Dipolar coupling data were used to further substantiate this assignment; correlations between H-22a and H-20, between H-21 and H-23, and between H-21 and H-22a were observed in the ROESY spectrum. The ROESY data showed no correlation between H-23 and H-20.

A similar analysis was used to assign the relative stereochemistry of C-22−C-23. The large homonuclear coupling constant $^3J_{H22bH23}$

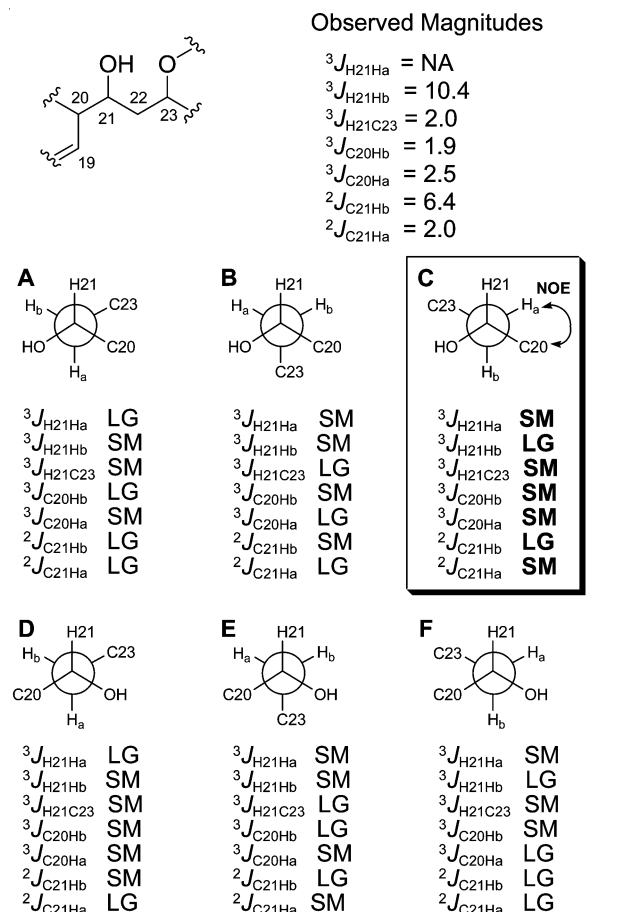

Figure 2. All possible rotamers for the *syn* and *anti* conformations for C-21−C-22 of 21-hydroxyoligomycin A. The boxed conformation corresponds to the rotamer with *J* values consistent with the measured homo- and heteronuclear coupling constants. The indicated NOE correlation provided supporting evidence for determination of the relative stereochemistry. NA represents values that could not be measured due to overlapping signals.

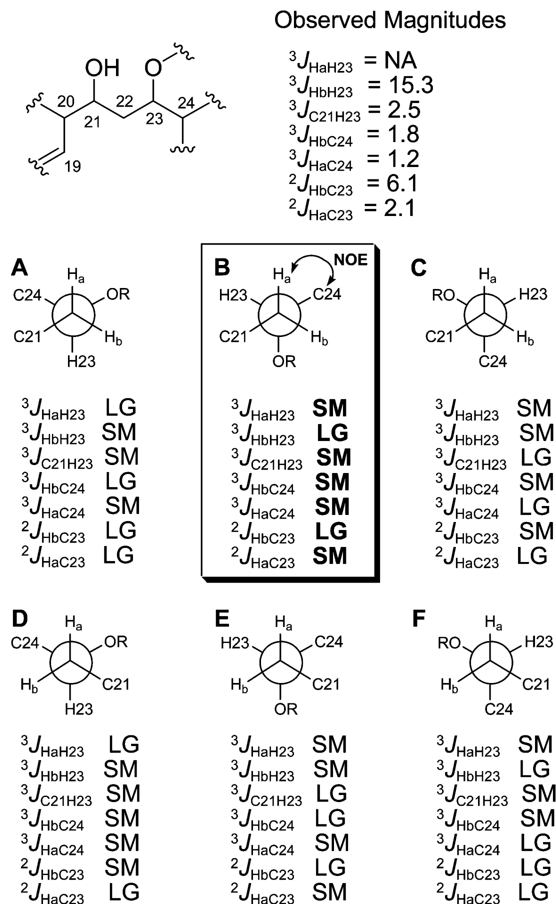

Figure 3. All possible rotamers for the *syn* and *anti* conformations for C-22−C-23 of 21-hydroxyoligomycin A. The boxed conformation corresponds to the rotamer with *J* values consistent with the measured homo- and heteronuclear coupling constants. The indicated NOE correlation provided supporting evidence for determination of the relative stereochemistry. NA represents values that could not be measured due to overlapping signals.

= 15.3 Hz indicated that H-22b and H-23 are in an *anti* relationship, consistent with either rotamer B or F (Figure 3). The $^3J_{HaC24}$ and $^2J_{HaC23}$ couplings can be used to distinguish between these two rotamers. On the basis of the observed coupling constants ($^3J_{HaC24}$ = 1.2 Hz and $^2J_{HaC23}$ = 2.1 Hz), the relative configuration of C-22−C-23 is as depicted in rotamer B. Additionally, an NOE between H-22a and H-24 supports this stereochemical assignment. On the basis of these data, the overall relative stereochemistry for these two centers was concluded to be 21*R**, 23*S**.

For completeness, the absolute structure of 21-hydroxyoligomycin A (**1**) was obtained by single-crystal X-ray diffraction. The 19 stereogenic centers in the molecule were unequivocally confirmed to be 4*S*, 5*R*, 6*S*, 8*R*, 9*R*, 10*S*, 12*R*, 13*S*, 14*R*, 20*S*, 21*R*, 23*S*, 24*R*, 25*R*, 26*S*, 27*R*, 30*S*, 31*S*, 33*R*. A stereoview of the molecular structure of 21-hydroxyoligomycin A (**1**) along with some of its associated chloroform and methanol molecules in the crystal lattice is provided in Figure 4, and a stereoview of its molecular conformation relative to those observed in the crystal structures of 44-homooligomycin B and rutamycin A is given in Figure 5.[23,24] A structure of oligomycin B is not included in the comparison since the coordinates from that 1972 study are not available.[25] Members of the oligomycin/rutamycin/cytovaricin family of antibiotics that do not contain a 26-membered ring were also excluded from the superposition. As shown in Figure 5, the core geometries for 21-hydroxyoligomycin A (**1**), 44-homooligomycin B, and rutamycin A are virtually identical. These observations are not surprising given the rigidity built into these molecules by the presence of a spiroketal unit and alkenyl and carbonyl functionalities. These X-ray results corroborate the notion mentioned above that a common absolute configurational assignment within this family of spiroketal macrolides is completely valid.

21-Hydroxyoligomycin A (**1**), 44-homooligomycin B, and rutamycin A crystallize in the triclinic space group *P*1, the orthorhombic space group $P2_12_12_1$, and the monoclinic space group $P2_1$, respectively, and with completely different unit cell constants and occluded solvents. Their *inter*molecular interactions with their associated solvent spheres are completely different, and the packing of these macrolides in their respective crystals shows no similarities at all. Yet, as indicated above, their molecular conformations are virtually identical. The prevailing thought based on these results must therefore be that the homologous core geometries and conformations observed for these macrolides are dictated by *intra*molecular forces, e.g., by geometrical constraints imposed by the rigid spiroketal, alkenyl, and carbonyl subunits, and by *intra*molecular hydrogen bonds. For example, the hydrogen bonds between the C-5 hydroxyl group and the C-7 carbonyl oxygen atom and between the C-12 and C-13 hydroxyl groups are conserved in all three structures. A third intramolecular hydrogen bond between the C-21 hydroxyl group and the spiroketal oxygen atom between C-23 and C-27 is also present in 21-hydroxyoligomycin A (**1**), but not in 44-homooligomycin B or rutamycin A. That these intramolecular hydrogen bonds are conserved in spite of concurrent but dissimilar intermolecular interactions suggests that they may be secondary contributors to the stability of the common observed conformation for these macrolides.

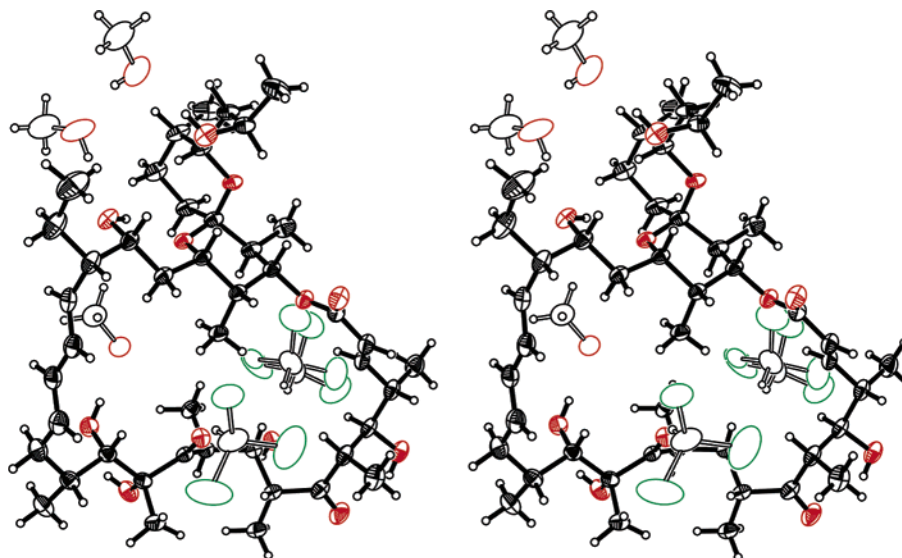

Figure 4. Stereoview of 21-hydroxyoligomycin A bis(chloroform) tris(methanol) solvate. The nonhydrogen atoms are depicted with 35% probability ellipsoids, and any dotted lines indicative of hydrogen-bonding interactions have been omitted for clarity.

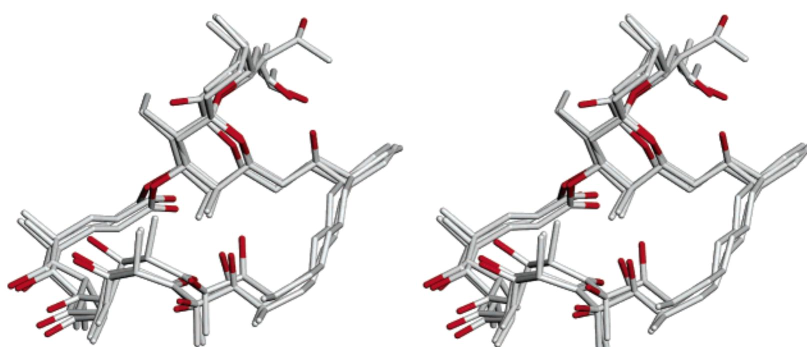

Figure 5. Stereoview of the superposition of 21-hydroxyoligomycin A, 44-homooligomycin B, and rutamycin A. Solvent molecules and hydrogen atoms have been omitted for clarity.

Not unexpectedly, conformational flexibility is observed in the dangling hydroxypropyl substituent on C-31; for example, the positioning of this substituent is quite similar in 21-hydroxyoligomycin A (**1**) and rutamycin A, but different than that observed in 44-homooligomycin B. That they are similar in 21-hydroxyoligomycin A (**1**) and rutamycin A is purely happenstance and no conformational preference is implied. The hydroxypropyl substituent is leashed by a series of hydrogen bonds to the C-21 hydroxyl group in 21-hydroxyoligomycin A (**1**) and is pinned to the C-1 carbonyl oxygen atom by a completely different series of hydrogen bonds in rutamycin A. That both networks of hydrogen bonds yield essentially the same orientation for the hydroxypropyl group is considered to be a chance occurrence.

Finally, a brief discussion of the chloroform molecules incorporated into the crystal lattice of 21-hydroxyoligomycin A (**1**) is provided. The positioning of the two crystallographically independent chloroform molecules relative to the macrolide is illustrated in Figure 4. It is worth noting that the C-11 carbonyl oxygen atom in 44-homooligomycin B and rutamycin A does not participate in any intermolecular close contacts. Therefore, this unutilized and open acceptor site in the macrolide ring of 21-hydroxyoligomycin A (**1**) could be exploited and tagged with a heavy atom label, e.g., via hydrogen bonding to a chloroform donor. We are pleased to report that that did indeed transpire, allowing for the complete and absolute structure determination of 21-hydroxyoligomycin A (**1**) by anomalous dispersion methods. A second chloroform molecule in the lattice was unexpected and is disordered and hydrogen bonded to the C-9 hydroxyl group. The range of observed H···O and C···O distances are 2.03−2.21 and 3.00−3.12 Å, respectively, versus 2.72 and 3.22 Å for the sum of the van der Waals radii for each pair.

Of course, the most significant point in regard to the 21-hydroxyoligomycin A (**1**) X-ray structure is that unlike earlier studies by others, which lacked an anomalous scatterer or a chiral auxiliary, to our knowledge, this is the first absolute structure determination of an oligomycin based purely on single-crystal X-ray crystallography. As such, a completely independent and unbiased structure is now available to complement those derived by chemical degradation and spectroscopic means.

Experimental Section

General Experimental Procedures. The optical rotation was measured using a Perkin-Elmer 241 polarimeter. The UV spectrum was recorded on a Hewlett-Packard 8453 UV−vis spectrophotometer. The IR spectrum was recorded on a Thermo Nicolet Nexus 470 FT-IR ESP spectrometer using ATR. ^{1}H and 2D NMR experiments were performed on a Bruker DRX500 spectrometer equipped with a Bruker 5 mm TXI CryoProbe. A Bruker Avance 400 spectrometer equipped with a Bruker 3 mm probe operating at 100 MHz was used to acquire ^{13}C NMR data. NMR spectra were recorded using $CDCl_3$ at 298 K, and the chemical shifts were referenced relative to the corresponding solvent signals (δ_H 7.27 for ^{1}H NMR and δ_C 77.23 for ^{13}C NMR). Mass spectrometric data were acquired on Bruker APEX II 9.4T FTMS. X-ray crystallographic data were collected on a Nonius KappaCCD diffractometer equipped with an MSC X-stream cryostream and Mo Kα radiation (λ = 0.71073 Å). Oligomycin A was obtained commercially from Sigma-Aldrich.

Biological Material. The actinomycete *Streptomyces cyaneogriseus* ssp. *noncyanogenus* is maintained in the culture collection of Wyeth, Pearl River, New York, as culture number LL-F28249. A voucher of this microorganism with accession number NRRL 15773 has been deposited with the Patent Culture Collection Laboratory, Northern Regional Research Center, U.S. Department of Agriculture, Peoria, Illinois 61604. Large-scale culturing of the actinomycete *S. cyaneogriseus* ssp. *noncyanogenus* (LL-F28249) followed previously published methods for seed production.[26] Ten liters of the second-stage seed culture was used to inoculate each of two 300 L tanks containing the sterilized production medium: dextrin 1.0%, soya peptone 1.0%, molasses 2.0%, calcium carbonate 0.1% in water. The fermentation was conducted at 30 °C with a sterile air flow of 30 L per min, with an impeller speed of 500 rpm. The whole broth was harvested after 118 h.

Extraction and Isolation. A total of 450 L of whole fermentation broth was mixed with Celite and filtered, and the resultant filter cake was extracted with methanol. The methanol was concentrated under reduced pressure, yielding an aqueous residue. This aqueous suspension was partitioned using methylene chloride. Evaporation of the organic layer left a syrup, which was treated with hexane to remove nonpolar materials, yielding 9 g of hexane-insoluble residue. The 9 g of crude complex was solubilized in 50 mL 10:10:1 methylene chloride−hexane−methanol and charged onto an LH-20 column (10 × 110 cm). The column was developed at a flow rate of 5−8 mL/min. An initial forerun of 2150 mL was discarded, and fractions (400 mL) were then collected. Fractions 9−12 were combined and concentrated to yield a residue containing 21-hydroxyoligomycin A (**1**). This residue was further purified by reversed-phase HPLC using a YMC ODS-A column (30 × 250 mm; S-5 μm; 12 nm) and 85% acetonitrile in water isocratic (flow rate = 12 mL/min; t_R = 22.2 min).

21-Hydroxyoligomycin A (1): $[\alpha]^{26}_{D}$ −49 (*c* 0.35, methanol); UV (MeOH) λ_{max} 225, 232, 241 nm; IR (KBr) ν_{max} 3457, 2967, 2937, 2844, 1700, 1458, 1379, 1283, 982 cm^{-1}; ^{1}H NMR (Table 1); ^{13}C NMR (Table 1); HRMS *m/z* 807.5274 (calcd for $C_{45}H_{75}O_{12}$, 807.5253).

Absolute Structure of 21-Hydroxyoligomycin A (1). Compound **1** was recrystallized from a solvent mixture of methanol, chloroform, and hexanes as colorless needles of $C_{45}H_{74}O_{12}\cdot 2CHCl_3\cdot 3CH_3OH$. Anomalous dispersion by the chlorine atoms allowed the absolute configurations for the 19 chiral centers in the molecule to be assigned with certainty by a single-crystal X-ray diffraction experiment. Crystal data: Triclinic, $P1$ (No. 1), a = 10.0874(2) Å, b = 12.5229(2) Å, c = 13.6632(2) Å, α = 65.648(1)°, β = 88.150(1)°, γ = 86.102(1)°, V = 1568.77(5) Å^3, Z = 1, and $R[F^2 > 2\sigma(F^2)] = 0.0603$ with the Flack absolute structure parameter being x = 0.01(6). Full experimental details and results are available upon request.[27]

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Supporting Information Available: ^{1}H and ^{13}C NMR spectra of compound **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(27) Crystallographic data for the 21-hydroxyoligomycin A bis(chloroform) tris(methanol) solvate reported in this paper have been deposited with the Cambridge Crystallographic Data Centre. Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB2 1 EZ, UK (fax: +44-(0)1223-33603.3 or e-mail: deposit@ccdc.cam.ac.uk).

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