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ORIGINAL ARTICLE

The first examples of chemical modification of oligomycin A

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The first examples of chemical modification of antibiotic oligomycin A are described. The interaction of oligomycin A with hydroxylamine yielded six-membered nitrone annelated with the antibiotic at the positions 3,4,5,6,7. The reaction with 1-aminopyridinium iodide in pyridine led to pyrazolo[1,5-a]pyridine conjugated with the antibiotic at the positions 2 and 3 (product of addition to the C_2–C_3 double bond followed by spontaneous oxidation). The structures of the compounds obtained were supported by NMR and mass spectrometry methods including the ^{15}N-labeling of compounds.
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Keywords: 1-aminopyridine; oligomycin A; oligomycin A annelated with 2,3,4,5-tetrahydropyridine N-oxide; oligomycin A annelated with pyrazolo[1,5-a]pyridine; six-membered nitrone

INTRODUCTION

Oligomycins are cytotoxic macrolides that contain a 26-membered α,β-unsaturated lactone with a conjugated diene fused to a bicyclic spiroketal ring system. These compounds inhibit oxidative phosphorylation in mitochondria by preventing ATP synthesis. Their mode of action involves the decoupling of the F_0 and F_1 factors of mitochondrial ATPase responsible for facilitating proton transfer through the inner mitochondrial membrane.[1,2] The enzymatic complex F_0F_1 ATP synthase can be considered as a target for antitumor or anti-infection therapy.[3,4] Oligomycins display a variety of significant biological activities; in addition to the specific inhibition of mitochondrial ATPase, strong anti-actinobacterial,[4] antifungal effects[5] and antitumor[1,6] actions have been recorded. Oligomycins are among the topmost cell line selective agents; they block P-glycoprotein activity and trigger apoptosis in doxorubicin-resistant HepG2 cells.[7]

The oligomycin antibiotic complex was initially isolated in 1954 from a strain of *Streptomyces diastatochromogenes*.[2] The complex consists of variable proportions of three major components A, B and C-oligomycins depending on the strain and culture conditions.[5] Later, from various *Streptomyces* strains, oligomycins D, T, F and others were isolated and identified.[6,8–10] The structures and absolute configuration of oligomycins A and C were established by chemical correlation of their individual degradation products with those derived from rutamycin;[11,12] however, no data on chemically modified derivatives of oligomycins have been published until now.

RESULTS AND DISCUSSION

Interaction of oligomycin A with hydroxylamine and 1-aminopyridine

Availability of oligomycin A in substantial quantities by fermentation provides the basis for the expedient investigation of its mode of action and improvement of its therapeutic potential through direct chemical modification. To this end, we report the first examples of chemical modification of oligomycin A. The strain producer of the antibiotic *S. avermitilis* NICB62 was used for biosynthesis and isolation of oligomycin A.

With the aim of exploring the reactivity of functional groups of oligomycin A that might open the way to the preparation of more selective antitumor or anti-infective oligomycin A derivatives, we first studied the interaction of the antibiotic with carbonyl-specific reagents. By the interaction of the antibiotic with hydroxylamine hydrochloride or 1-aminopyridinium iodide in pyridine, we managed to isolate novel derivatives **2** and **3a**, respectively. The structures of novel compounds **2** and **3a** were determined on the basis of HR-electrospray ionization (ESI)-MS data and with the use of 1D (^{1}H and ^{13}C) and 2D NMR correlation spectra (^{1}H-^{1}H ^{1}COSY and ^{1}H-^{13}C HETCOR). Spectral parameters of these compounds were compared with those of oligomycin A obtained in this study and with literature data (Table 1).[8,13,14]

To elucidate the structures of novel derivatives, their ^{15}N-labeled analogs ^{15}N-**2** and ^{15}N-**3** were synthesized with the use of ^{15}N-hydroxyl amine or 1-^{15}N-aminopyridine, respectively. For the ^{15}N-labeled compounds, 1-D (^{1}H and ^{13}C) spectra were measured and the coupling constants $J_{15N,1H}$ and $J_{15N,13C}$ were determined (Table 2).

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Table 1 ^{13}C and ^{1}H NMR spectra of compounds 1, 2 and 3a (δ_C, δ_H p.p.m., J Hz) in $CDCl_3$[a]

	1					***2***					***3a***				
C no.	δ_C			δ_H	$J_{H,H}$	δ_C			δ_H	$J_{H,H}$	δ_C			δ_H	$J_{H,H}$
1	165.02	O-CO				169.45	O-CO				164.09	O-CO			
2	122.61	CH	sp^2	5.80dd	15.6, 0.7	34.15	CH_2	sp^3	2.81dd; 2.68dd		99.77	Cq	sp^2		
3	148.29	CH	sp^2	6.62dd	15.6, 10.1	68.85	CH	sp^3	3.92m	9.8, 9.3, 3.1, 2.0[b]	163.53	Cq	sp^2		
4	40.06	CH	sp^3	2.36tq	10.0, 6.6	39.76	CH	sp^3	1.95m	~10.0, ~10.0	35.38	CH	sp^3	3.85dq	2.0, 7.2
5	72.88	CH	sp^3	3.75dd	10.1, 1.3	76.45	CH	sp^3	3.18dd	10.1, 6.7	73.89	CH	sp^3	4.16dd	8.3, 2.0
6	46.38	CH	sp^3	2.70dq	1.3, 7.4	44.23	CH	sp^3	2.33ddq	~7.0, 2.0, 7.0	48.44	CH	sp^3	3.04dq	8.2, 7.0
7	220.16[c]	CO				153.70	C=N				217.76[c]	CO			
8	45.63[d]	CH	sp^3	2.74dq	3.0, 7.1	38.91	CH	sp^3	2.54q	7.0	47.27[d]	CH	sp^3	2.80dq	1.8, 6.8
9	72.57	CH	sp^3	3.94dd	8.6, 3.1	74.11	CH	sp^3	3.88d	8.3	71.70	CH	sp^3	4.25dd	8.5, 1.8
10	41.88[d]	CH	sp^3	3.59dq	8.6, 6.9	44.12	CH	sp^3	3.13dq	8.3, 6.8	42.97[d]	CH	sp^3	3.23dq	8.4, 6.8
11	219.93[c]	CO				217.22	CO				215.93[c]	CO			
12	82.91	C_q-O	sp^3			82.35	C_q-O	sp^3			82.53	C_q-O	sp^3		
13	72.15	CH	sp^3	3.89d	1.9	70.48	CH	sp^3	3.89bs		70.70	CH	sp^3	4.00bs	
14	33.41	CH	sp^3	1.88m		33.64	CH	sp^3	1.85m		33.60	CH	sp^3	1.86m	
15	38.33	CH_2	sp^3	2.17bd; 1.98dt		38.20	CH_2	sp^3	2.16m, ~2.05m		38.35	CH_2	sp^3	2.13m; ~2.03m	
16	129.30	CH	sp^2	5.42ddd	14.8, 10.5, 4.1	130.46	CH	sp^2	5.38ddd	14.6, 10.3, 4.1	129.56	CH	sp^2	5.48m	
17	132.30	CH	sp^2	6.00ddd	14.7, 10.4, 1.4	133.14	CH	sp^2	6.00ddd	14.6, 10.4, 1.5	133.61	CH	sp^2	6.02m	
18	130.19	CH	sp^2	5.90dd	14.9, 10.5	130.39	CH	sp^2	5.95dd	14.2, 10.4	129.75	CH	sp^2	6.02m	
19	137.67	CH	sp^2	5.21dd	14.8, 9.6	135.80	CH	sp^2	5.39dd	14.2, 10.0	138.29	CH	sp^2	5.36m	
20	45.94	CH	sp^3	1.85m		43.56	CH	sp^3	1.96m		44.13	CH	sp^3	1.98m	
21	31.38	CH_2	sp^3	1.52m; 1.35m		25.95[c]	CH_2	sp^3	1.47m; 1.38m		32.01	CH_2	sp^3	~1.50m; ~1.40m	
22	30.90	CH_2	sp^3	1.59ddd; ~1.02m		25.87[c]	CH_2	sp^3	1.50m; 1.35m		28.70	CH_2	sp^3	1.54m; 1.38m	
23	68.95	CH	sp^3	3.78ddd	9.8, 2.7, 24	68.88	CH	sp^3	3.68m		69.67	CH	sp^3	3.89td	5.3, 2.2
24	35.74	CH	sp^3	2.11ddq	5.0, 2.2, 6.9	34.21	CH	sp^3	2.03m		35.15	CH	sp^3	2.26ddq	4.6, 2.0, 7.0
25	76.10	CH	sp^3	4.91dd	11.4, 5.0	75.13	CH	sp^3	5.09dd	11.5, 4.3	76.32	CH	sp^3	5.23dd	11.5, 4.7
26	37.61	CH	sp^3	1.78dq	11.4, 6.6	37.93	CH	sp^3	1.69dq	11.5, 6.7	37.52	CH	sp^3	2.00dq	11.5, 6.7
27	99.11	O-C-O	sp^3			99.21	O-C-O	sp^3			99.24	O-C-O	sp^3		
28	25.88	CH_2	sp^3	1.90m; 1.23m		25.80	CH_2	sp^3	1.86m; 1.20m		25.78	CH_2	sp^3	1.92m; 1.27m	
29	26.41	CH_2	sp^3	2.07m; 1.38m		26.48	CH_2	sp^3	2.10m; 1.35m		26.52	CH_2	sp^3	2.15m; 1.40m	
30	30.38	CH	sp^3	1.54m		30.38	CH	sp^3	1.52m		30.23	CH	sp^3	1.56m	
31	67.14	CH	sp^3	3.96dt	10.3, 2.5	67.00	CH	sp^3	3.96dt	10.6, 2.5	66.84	CH	sp^3	4.01dt	10.6, 2.5
32	42.46	CH_2	sp^3	1.55m, 1.25m		42.15	CH_2	sp^3	1.59ddd, 1.22m		41.97	CH_2	sp^3	1.60ddd; 1.28m	
33	64.57	CH	sp^3	4.00ddq	9.2, 3.1, 6.2	64.40	CH	sp^3	4.02ddq	9.3, 3.0, 6.2	64.14	CH	sp^3	4.09ddq	9.3, 2.9, 6.2
34	24.66	CH_3	sp^3	1.213d	6.2	24.67	CH_3	sp^3	1.195d	6.3	24.81	CH_3	sp^3	1.217d	6.2
35	17.84	CH_3	sp^3	1.159d	6.6	15.11	CH_3	sp^3	1.084d	6.6	12.97	CH_3	sp^3	1.385d	7.2
36	8.21	CH_3	sp^3	1.047d	7.3	16.31	CH_3	sp^3	1.261d	7.0	14.04	CH_3	sp^3	1.137d	7.0
37	9.16[e]	CH_3	sp^3	1.011d	7.0	7.48	CH_3	sp^3	1.063d	6.9	9.67[e]	CH_3	sp^3	1.005d	6.8
38	13.99[e]	CH_3	sp^3	1.085d	6.9	16.87	CH_3	sp^3	1.178d	6.8	16.37[e]	CH_3	sp^3	1.295d	6.8
39	20.92	CH_3	sp^3	1.110s		21.07	CH_3	sp^3	1.270s		21.73	CH_3	sp^3	1.260s	
40	14.39	CH_3	sp^3	0.977d	6.6	14.23	CH_3	sp^3	0.947d	6.8	14.38	CH_3	sp^3	0.966d	6.8
41	28.42	CH_2	sp^3	~1.35m, 1.25m		29.10	CH_2	sp^3	1.38m, ~ 1.20m		26.42	CH_2	sp^3	1.42m, ~ 1.20m	
42	11.98	CH_3	sp^3	0.799t	7.4	12.08	CH_3	sp^3	0.785t	7.3	11.92	CH_3	sp^3	0.799t	7.4
43	5.97	CH_3	sp^3	0.822d	6.9	6.06	CH_3	sp^3	0.730d	6.8	5.55	CH_3	sp^3	0.894d	6.9
44	11.67	CH_3	sp^3	0.950d	6.6	11.46	CH_3	sp^3	0.890d	6.6	11.74	CH_3	sp^3	1.043d	6.6
45	11.13	CH_3	sp^3	0.884d	6.9	11.13	CH_3	sp^3	0.867d	7.0	11.40	CH_3	sp^3	0.897d	7.0
2′											141.11	C_q	sp^2		
3′											118.96	CH	sp^2	7.97ddd	8.8, 1.4, 1.0
4′											127.56	CH	sp^2	7.38ddd	8.8, 6.9, 1.1
5′											103.42	CH	sp^2	6.91dt	1.4, 6.9
6′											128.95	CH	sp^2	8.44dt	6.9, 1.0

[a]In the literature there are discrepancies in the assignments of ^{13}C and ^{1}H signals of segment F2 for oligomycin A[8] and its analogous oligomycins B and C.[13] For compound **2** the assignment of signals in segment F2 is unambiguous because of the presence of spin coupling constants $^{2}J_{15N,\ 8\text{-}C}$=3.5 Hz and $^{3}J_{15N,\ 8\text{-}H}$=4.2 Hz. For compound **3a** as well as for the starting oligomycin A, there is a possibility of interchangeable assignment of C-8 and C-10 and the atoms connected with them; however, it does not influence the deduction of **3a** structure.
[b]Spin coupling values are measured in $CDCl_3/C_6D_6$, 2:3 mixture.
[c,d,e]Reverse assignments of signals are possible.

Table 2 Spin coupling values $J_{15N,\ 13C}$ and $J_{15N,\ 1H}$ (Hz) in ^{13}C and ^{1}H NMR spectra of ^{15}N-2 and ^{15}N-3a in $CDCl_3$

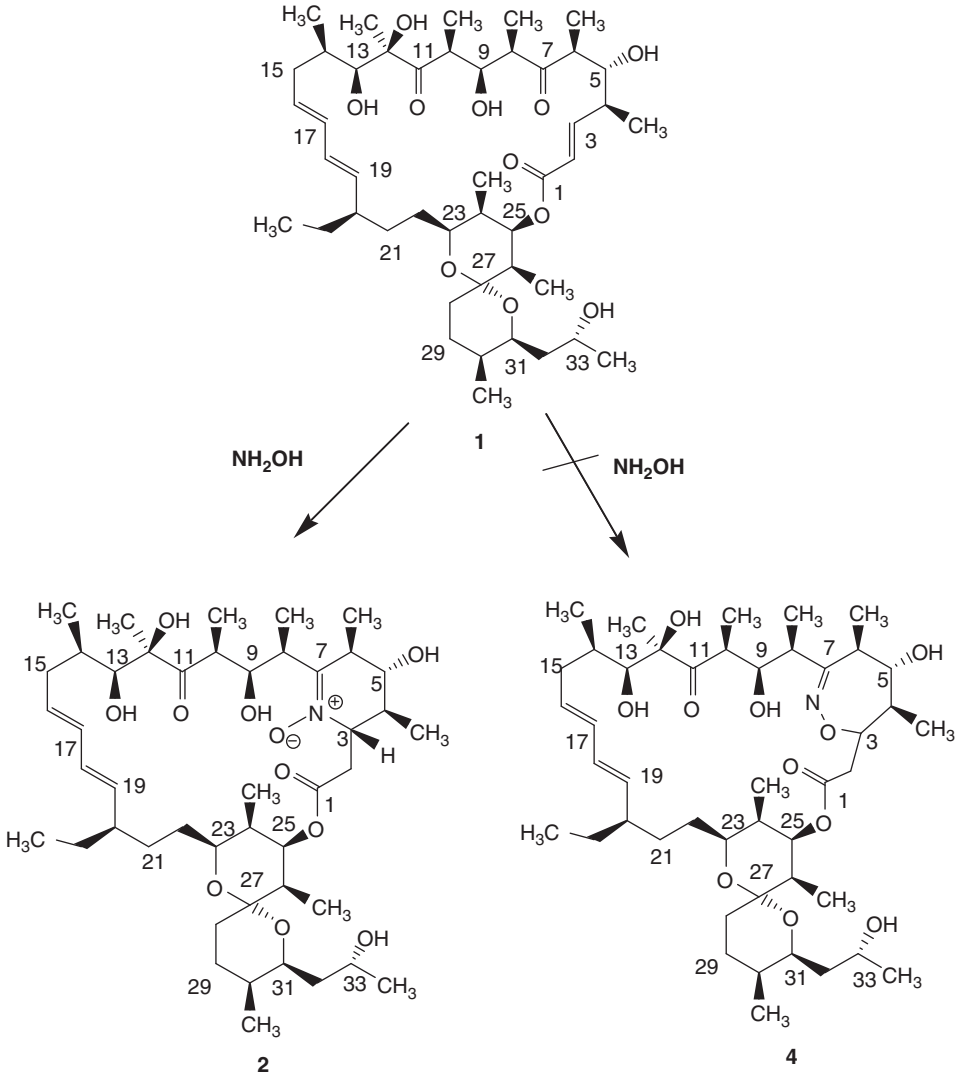
	^{15}N-2				*^{15}N-3a*			
C no.	δ_C	$J_{15N,\ 13C}$	δ_H	$J_{15N,\ 1H}$	δ_C	$J_{15N,\ 13C}$	δ_H	$J_{15N,\ 1H}$
1	169.45	$^{3}J\leqslant1.5$			164.09	$^{3}J\leqslant1.5$	—	—
2A	34.15	$^{2}J\leqslant1.5$	2.81	$^{3}J=4.0$	99.75	$^{2}J\leqslant2.0$	—	—
2B			2.68	$^{3}J=2.3$				
3	68.85	$^{1}J=7.0$	3.92	$^{2}J\leqslant1.0$	163.53	$^{1}J\leqslant1.5$	—	—
4	39.76	$^{2}J\leqslant1.5$	1.95	$^{3}J\leqslant1.0$	35.37	$^{2}J=5.4$	3.85	$^{3}J=4.0$
5	76.45	$^{3}J=2.0$	3.18	$^{4}J\leqslant1.0$	73.89	$^{3}J\leqslant1.5$	4.16	$^{4}J\leqslant1.0$
6	44.23	$^{2}J\leqslant1.5$	2.33	$^{3}J=4.3$	48.44	$^{4}J\leqslant1.5$	3.04	$^{5}J\leqslant1.0$
7	153.70	$^{1}J=19.8$	—	—				
8	38.91	$^{2}J=3.5$	2.54	$^{3}J=4.2$				
9	74.11	$^{3}J=1.7$	3.88	$^{4}J\leqslant1.0$				
10	44.12	$^{4}J\leqslant1.5$	3.13	$^{5}J\leqslant1.0$				
35	15.11	$^{3}J=1.8$	1.084	$^{4}J\leqslant1.0$	12.97	$^{3}J\leqslant1.5$	1.385	$^{4}J\leqslant1.0$
6′					128.95	$^{2}J=6.0$	8.44	$^{3}J=1.0$

We showed in this study that compound **2** was a six-membered nitrone (2,3,4,5-tetrahydropyridine N-oxide) annelated with the macrolide cycle (Scheme 1), and that compound **3a** was pyrazolo[1,5-a]pyridine annelated with the oligomycin A framework (Scheme 3). Similarly, the interaction of oligomycin A with 1-amino-4-methylpyridine yielded the analogous structure (**3b**).

Elucidation of the structure of nitrone annelated with oligomycin A

Cyclic nitrones result from the intramolecular 1,3-azaprotio cyclotransfer reaction of the oxime group with the olefinic bond. The reaction requires the presence of the terminal olefinic electron-withdrawing ester group CO_2R. Also, the product(s) of reaction were shown to depend on the space-filling capacity of substituents.[15,16] For certain oximes, the two modes of reaction illustrated in Scheme 2 are in competition, and the preferred reaction path in any given case will be that of lowest energy.[17]

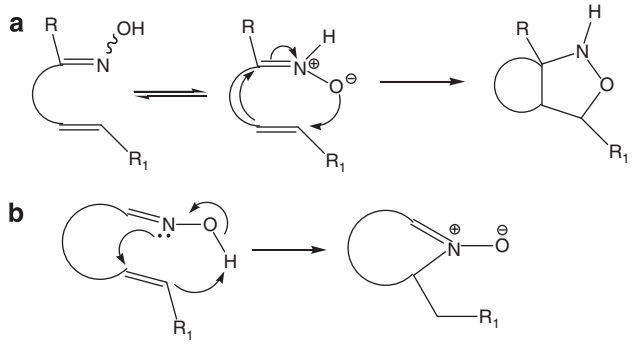
In our case paths, (a) and (b) would lead to structures **4** or **2,** respectively, with the same molecular formula $C_{44}H_{75}NO_{11}$ determined by HR-ESI-MS.



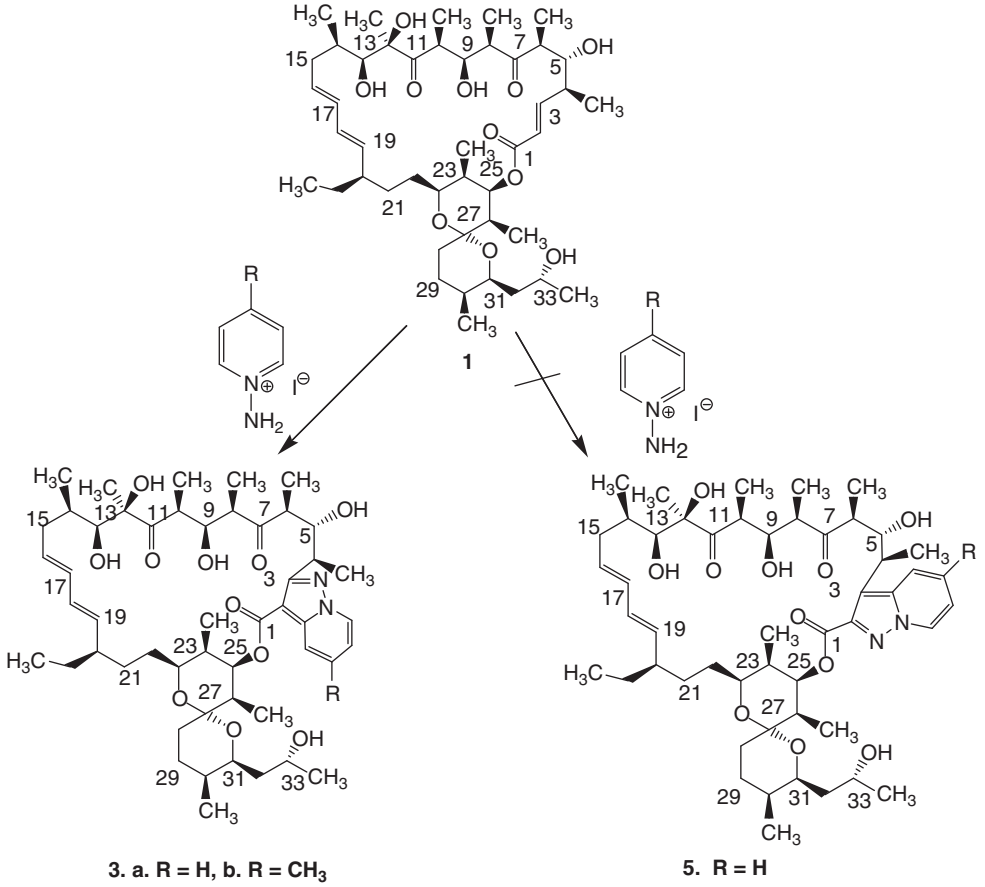
Scheme 1 Interaction of oligomycin A with hydroxylamine.

The detailed NMR investigation of compound **2** and its ^{15}N-labeled derivative was performed to elucidate the structure of this compound and to select between nitrone **2** and the otherwise possible 1,2-oxazepin **4**. In our NMR study of compound **2**, we followed the approach that was described by Laatsch *et al*.[8] and consisted in the study of the structural fragments of the antibiotic. There are four structural fragments in oligomycin A and its novel derivatives **2** and **3**, which are characterized by relatively strong spin interactions between the geminal and vicinal hydrogen atoms F1 (C2–C6), F2 (C8–C10), F3 (C13–C26) and F4 (C28–C34).

The comparison of chemical shifts of ^{1}H and ^{13}C atoms in segments F3 (C13–C26) and F4 (C28–C34) in compound **2** and oligomycin A shows that the differences in these segments are connected only with the differences in mutual dispositions of the atoms but not with the changes in atom hybridization or changes in the number of atoms. This conclusion is supported by rather close values of δC for the majority of carbon atoms of the structural segments F3 and F4 of **1** and **2** (differences $\leqslant 1.5$ p.p.m.). The exclusions are presented by C_{20} ($\Delta\delta=2.4$ p.p.m.), C_{21} ($\Delta\delta=5.4$ p.p.m.) and C_{22} ($\Delta\delta=5.0$ p.p.m.); however, even in these cases, the type of hybridization and the number of C–H and C–C bonds in compounds **1** and **2** are similar. It is also true of parameters of the ^{1}H NMR spectra of compounds **1** and **2**. Contrary to the segments F3 and F4, the parameters of the ^{1}H and ^{13}C NMR spectra of segments F1 and F2 in compounds **1** and **2** are substantially different. First of all, these differences show the presence of the C=N bond and the singular C2–C3 bond in compound **2** instead of C=O and double C2=C3 bond in compound **1**. The elucidation of the structure of 2,3,4,5-tetrahydropyridine N-oxide annelated with the macrolide cycle at the positions 3,4,5,6,7 was also based on the comparison of the ^{1}H and ^{13}C NMR spectra of compounds **2** and ^{15}N-**2** (Table 2). The value of $^{3}J_{15N,\ 1H\text{-}6}=4.3$ Hz supports the bond C_7=N and permitted us to exclude any structure with the C_{11}=N bond. The annelated 1,2-oxazepine structure that might be formed at the interaction of an unsaturated carbonyl compound with hydroxylamine is in contradiction with the values of constants of spin interactions $^{3}J_{15N,\ 1HA\text{-}2}=4.0$ Hz and $^{3}J_{15N,\ 1HB\text{-}2}=2.3$ Hz. For the same reason, we excluded from our consideration other cyclic structures with the N–O bond or acyclic structures with N–OH moiety; the latter must be excluded because



Scheme 2 Dipole formation from oximes; (**a**) intramolecular oxime–olefin cycloaddition and (**b**) intramolecular 1,3-azaprotio cyclotransfer reaction (APT).



Scheme 3 Interaction of oligomycin A with 1-aminopyridinium iodide in pyridine.

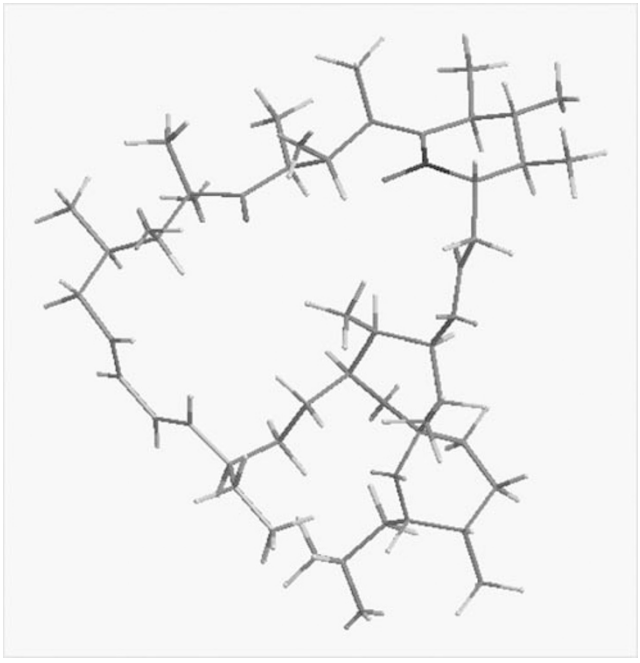


Figure 1 Three-dimensional structure of compound **2**.

of the presence of spin interaction between ^{15}N=C-7 and C-3 ($^{1}J_{15N, C-3}$=7.0 Hz). Structure **2** is also well supported by the high value of the constant $^{1}J_{15N, C-7}$=19.8 Hz[18] and the value of the constant $^{5}J_{H-3, H-6}$=2.0 Hz, which can be considered as an analog of homoallylic interaction.

The formation of a tetrahydropyridine cycle in compound **2** results in the formation of a new asymmetric atom at C-3. The high value of the constant $^{3}J_{H-3, H-4}$=10 Hz suggests that the atoms H-3 and H-4 are *trans*-oriented in six-membered nitrone and that the C3 atom has an *S* configuration (Figure 1).

Elucidation of the structure of pyrazolo[1,5-a]pyridine conjugated with oligomycin A

An unexpected product of the intermolecular cycloaddition to the C_2–C_3 double bond followed by simultaneous dehydrogenation (**3**a) was formed by the interaction of 1-aminopyridinium iodide with **1** in pyridine. The carbonyl groups did not participate in this reaction (Scheme 3).

It has been shown previously that the 1,3-dipolar cycloaddition of polymer-bound alkynes to azomethine imines produced substituted pyrazolo[1,5-a]pyridine derivatives.[19] However, we could not find the examples of the interaction of 1-aminopyridine with the double bond-containing compounds followed by spontaneous dehydrogenation. Still, there are examples of the interaction of 1-aminopyridine with double bond-containing compounds substituted at the double bond with groups that are split off in the reaction producing pyrazolo[1,5-a]pyridine derivatives.[20] Formation of pyrazolo[1,5-a] pyridine derivatives involves a regioselective [3+2] cycloaddition of 1-aminopyridine to alkenes or alkynes.

To help elucidate the structure of **3a**, 1-$^{15}NH_2$-pyridine was prepared from $^{15}NH_2OH$ and pyridine, producing ^{15}N-**3a** with **1** in pyridine**.** The NMR data for 1-^{15}N-aminopyridine are presented in Table 3. 1-Amino-4-methylpyridine gave 5-methylpyrazolo[1,5-a] pyridine annelated with the macrolide (**3b**) by the interaction with **1** in similar conditions. The structures of **3a** and **3b** were supported by mass spectrometry and NMR spectroscopy. We failed to prepare a product of the interaction of 1-amino-2-methylpyridine with oligomycin A, supposedly because of steric hindrances.

Table 3 Chemical shifts δ_C and δ_H (p.p.m.) and spin coupling constants $J_{15N, 13C}$ and $J_{15N, 1H}$ (Hz) in ^{13}C and ^{1}H NMR spectra of 1-^{15}N-aminopyridinium iodide in DMSO-d_6

C no.	δ_C	$J_{15N, 13C}$	δ_H	$J_{15N, 1H}$
2,6	138.37	2.0	8.77m	1.3
3,5	128.31	⩽1.0	8.03m	⩽0.5
4	139.86	⩽1.0	8.28m	⩽0.5
1-$^{15}NH_2$			8.46bd	73.0

The molecular formulas of compounds **3a** and **3b** were found by HR-ESI-MS to be $C_{50}H_{76}N_2O_{11}$ and $C_{51}H_{78}N_2O_{11}$, respectively. The determination of the structure of compound **3a** was similarly based on the comparison of hybridization and the number of hydrogen atoms connected with similar atoms in compounds **1** and **3**a (Table 1). This comparison shows that serious differences are present only for atoms C2 and C3, which are both sp^2 hybridized in **3a** and **1** but deprotonated in **3a**. C1 and other carbon atoms of the fragment F1, excluding C2 and C3, retain their type of hybridization (sp^2 for C1, sp^3 for C4, C5, C6) and the number of hydrogen atoms connected with them (0 for C1, 1 for each of C4, C5, C6). However, the dramatic differences between the spin coupling constants for similar hydrogen atoms in **1** and **3** show big differences in the steric structures of fragment F1 in oligomycin A and **3a**. NMR spectral parameters of fragments F3 and F4 are close in compound **3a** and oligomycin A. All carbon atoms of the 1-aminopyridine moiety in **3a** retain the starting sp^2 type of hybridization, but in **3a** one of the carbon atoms around 1-N is deprotonated, and the symmetry presented in the starting 1-aminopyridine is absent (atoms in positions 2/6 and 3/5 are not equivalent). The presence of two carbonyl groups in compound **3a** is supported by the presence of ^{13}C NMR signals at δ 217.76 and 215.93 p.p.m. In accordance with Ms data, it is suggested that compound **3a** has a structure of pyrazolo[1,5-a]pyridine annelated at the C_2–C_3 bond with the macrolide cycle. The selection between the possible structures **3a** and **5** was based on the investigation of NMR spectra of ^{15}N-**3**a (Table 3). The interaction between ^{15}N of the pyrazolopyridine moiety and C4-H of the macrolide cycle ($^{3}J_{15N, 4-H}$=4.0 Hz) supports the structure of **3a** as this coupling constant value is incompatible with the interaction through four bonds present in structure **5**.

The low value of the $^{1}J_{15N,13C}$ constant is in agreement with literature data. In aza-arenes, the direct spin coupling constant $^{1}J_{15N,13C}$ is close to 0 Hz, being both positive or negative.[18] Using the published data, we can compare this coupling constant with the analogous constant $^{1}J_{15N,13}$C in oximes (C=N-O), which has a rather small absolute value (0.5–5 Hz).[18]

In conclusion, the product of the interaction of oligomycin A with 1-aminopyridinium iodide has the structure of pyrazolo[1,5-a] pyridine annelated with the macrolide at the C2–C3 bond. The product of the interaction of oligomycin A with 1-amino-4-methylpyridinium iodide is its C-methylated analog (**3b**). The significant differences in the NMR spectra of **3a** and **3b** can be registered only in the area of the signals of the pyridine nucleus (δ, p.p.m., J, Hz for hydrogen atoms of the pyridine cycle in compound **3b** are the following: 3′-H: δ 7.73bs; 4′-CH_3: δ 2.43s; 5′-H: δ 6.73dd, J=7.0, 1.8; 6′-H: δ 8.29, J=7.0).

Cytotoxic, antibacterial and antifungal properties and structure-activity relationships for oligomycin A derivatives are under investigation. Preliminary studies have shown that compounds **2**, **3a** and **3b** are less cytotoxic than the starting antibiotic. Although oligomycin A is highly active against *Aspergillus niger* (MIC=0.125 μg ml^{-1}) and has moderate activity against *Cfndida albicans* and *Cryptococcus humicolus,* compound **2** is practically devoid of antifungal and anti-yeast properties. Compound **3** is active against *A. niger* with MIC=2 μg ml^{-1}, and its anti-yeast properties are close to those of oligomycin A.

METHODS

Experimental section

Oligomycin A (**1**), with a purity of 95%, was elaborated in the Scientific Research Centre for Biotechnology of Antibiotics BIOAN, Moscow, using selection strain-producer *S. avermitilis NIC B62* with the level of oligomycin A production about 1 g l^{-1}. Fermentation was performed for 8 days at 28 °C in liquid medium. Isolation and purification were performed by extraction with acetone-hexane mixture followed by crystallization.

All reagents and solvents were purchased from Aldrich (St Louis, MO, USA), Fluka (St Louis, MO, USA) and Merck (Darmstsdt, Germany). All solutions were dried over sodium sulfate and evaporated under reduced pressure on a Buchi rotary evaporator at <35 °C (Buchi, Dietikon, Switzerland). The progress reaction products, column eluates and all final samples were analyzed by TLC. $^{15}NH_2OH.HCl$ was obtained from Aldrich. ^{15}N-1-aminopyridinium iodide was obtained by the method described from $^{15}NH_2OSO_3H$ and pyridine.[21] TLC was performed on Merck $G60F_{254}$ precoated plates. Reaction products were purified by column chromatography on Merck silica gel 60 (0.04–0.063 mm). The NMR spectra were recorded on a Unity+400 (Varian, Palo Alto, CA, USA) spectrometer at 400.0 MHz for nuclei ^1H, and at 100.6 MHz for nuclei ^{13}C. The NMR elucidation was made with the use of homo ^1H, ^1H (2D COSY) and hetero ^1H, ^{13}C (2D HETCOR) correlation as well as ^1H and ^{13}C 1D spectra of the antibiotic, its derivatives and their ^{15}N-labeled compounds. ESI mass spectra were recorded on a Finnigan MAT 900S spectrometer (Finnigan, Bremen, Germany). UV spectra were obtained on a UV/VIS double beam spectrometer UNICO in methanol. IR spectra were recorded on Thermo Nicolet iS10 (Thermo Scientific, Waltham, MA, USA). Optical rotation was measured on AA55 Polarimeter (Optical Activity, Huntingdon, UK).

*Synthesis of oligomycin A annelated with 2,3,4,5-tetrahydropyridine-1-oxide (**2**).* To a solution of oligomycin A (100 mg, 0.13 mmol) in pyridine (4 ml) was added $NH_2OH.HCl$ (80 mg, 1.1 mmol) and KOAc (10 mg, 0.1 mmol) and the reaction mixture was stirred at room temperature for 48 h. Then it was poured on ice, acidified by 1N HCl till pH 3, and the reaction product was extracted with EtOAc. The extract was washed with water and dried over Na_2SO_4. The reaction product was purified by column chromatography in $CHCl_3$-MeOH (25:1) to give 50 mg (53%) of compound **2** as a colorless amorphous solid with m.p. 142–148 °C. R_F 0.42 ($CHCl_3$/MeOH, 10:1). MW calculated for $C_{45}H_{75}NO_{11}$ 805.5340, observed in ESI mass spectrum 806.5330 $(M+H)^+$. UV spectrum, λ_{max} (MeOH): 232 (ε 12.193). IR (KBr) (cm^{-1}): 1730, 1700, 1458, 1384, 988. $[\alpha]_D^{20}$=+160° (C1, MeOH). ^{15}N-**2** was obtained similarly with the use of $^{15}NH_2OH.HCl$.

*Synthesis of oligomycin A annelated with pyrazolo[1,5-a]pyridine (**3a**).* To a stirred solution of oligomycin A (100 mg, 0.13 mmol) in pyridine (4 ml) was added 1-aminopyridinium iodide (80 mg, 0.36 mmol) and K_2CO_3 (60 mg, 0.43 mmol) and the reaction mixture was heated at 40 °C for 48 h. Then it was poured on ice, acidified by 1 N HCl till pH 3, and the reaction product was extracted with $CHCl_3$. The extract was washed with water to pH 7, dried over Na_2SO_4 and purified by column chromatography in hexane/aceton (4:1) to obtain colorless amorphous solid (38 mg, 42%). M.p. 92–94 °C, R_F 0.54 (hexane/aceton, 2:1). MW calculated for $C_{50}H_{76}N_2O_{11}$ 880.5449, observed in ESI mass spectrum 881.5461 $(M+H)^+$, 903.5160 $(M+Na)^+$. UV spectrum, λ_{max} (MeOH): 226 (ε 9.03), 301 (1.3). IR (KBr) (cm^{-1}): 1699, 1516, 1456, 988. $[\alpha]_D^{20}$=+140° (C1, MeOH). ^{15}N-**3a** was obtained similarly with the use of 1-$^{15}NH_2$-C_5H_5N.

Synthesis of oligomycin A annelated with 5′-methyl-pyrazolo[1,5-a] pyridine (3b). Compound **3b** was obtained from oligomycin A and 1-amino-4-methylpyridine by a similar method with 24% yield. M.p. 110–112 °C. MW calculated for $C_{51}H_{78}N_2O_{11}$ 894.5606, observed in ESI mass spectrum 895.573 $(M+H)^+$. UV spectrum, λ_{max} (MeOH): 297 (ε 6.9).
$[\alpha]_D^{20}$=+136° (C1, MeOH) ⌋

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